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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 11000-01 ODIK

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Physics of Ionic Channels and other Proteins with Aqueous Cavities

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	V.A. Parsegian	Guest Researcher	
Others:	S.M. Bezrukov	Visiting Scientist	LBM, NIDDK
	J.J. Kasianowicz	Guest Researcher	LBM, NIDDK
	D.C. Rau	Research Chemist	LBM, NIDDK
	R. Brutyan	Special Volunteer	LBM, NIDDK

COOPERATING UNITS (if any)
Office of Naval Research, Arlington, VA (Dr. I. Vodyanoy); University of Maryland, College Park, MD (Dr. M. Colombini); Monell Chemical Senses Center, Philadelphia, PA (Dr. A.M. Feigin)

LAB/BRANCH
Division of Intramural Research

SECTION
Biophysics Unit

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Channels made from the peptide alamethicin have been observed while subjected to the osmotic action of differently sized neutral polymers. It is possible not only to see the degree of penetration of the polymers into the channel from their osmotic action but also to follow the kinetics of motion of small polymers through the ionic channel.

These channels are sensitive to the phospholipid of the bilayer into which they are incorporated, in particular there is a strong correlation between the probability of high-conductance states and the tendency of the phospholipid to form non-lamellar structures.

Hofmeister effect is shown to apply to transport properties of ionic channels. Chaotropic anions bind to roflamycoin channels for longer times, increase their conductance and induce cationic selectivity according to their position in Hofmeister series.

In experiments on hemoglobin oxygenation, it is demonstrated that for weak binding reactions the osmotic contributions to binding can be as significant as the direct contribution from solute binding.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 11001-01 ODIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Physical Properties of DNA and DNA-Protein Complexes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	D.C. Rau	Research Chemist	OD, NIDDK
Others:	N.Yu. Sidorova	Guest Researcher	OD, NIDDK

COOPERATING UNITS (if any)

LPTB, NICHD (Dr. M. Garner); Towson State University, Towson, MD (Dr. R. Preisler); University of Nevada, Reno, NV (Dr. R. Harrington): LCB, NHLBI (Dr. E. Korn).

LAB/BRANCH

Division of Intramural Research

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Direct measurement of forces between molecules in condensed arrays show a dominating contribution from water structuring. Now measurements of the osmotic sensitivity of DNA-protein and DNA-drug complex formation in dilute solution are also showing a link between binding strength and structured water release. Binding constants for the specific association of galactose repressor with operator and for nonspecific binding differ by a factor of 10^4. This difference is reflected in the release of some 150 water molecules for operator binding compared with nonspecific association. Even much smaller binding energy differences of repressor to different operator sequences are seen as differences in water release. The binding constant of the gal repressor-O_E operator association is twice that of the repressor-O_I complex and is accompanied by the relative release of 6 water molecules. A connection between energy and water release is also seen for drug-DNA interactions. The binding constant of netropsin to an Eco RI site is 10 times stronger than to an Nde I site and also releases some 30 more water molecules.

An important role for a change in hydration accompanying a nucleic acid conformational transition has been uncovered. The sensitivity of the B-Z transition to water activity indicates that a difference in the number of solute excluding water molecules between the two conformations is central to the transition. Conditions in the cell may be sufficiently crowded to allow the Z-form to exist without other stresses.

Changes in the internal flexibility of Acanthamoeba myosin II minifilaments with ATP binding have been further characterized. Minifilaments with bound ATP are about 50 fold more flexible than with either no bound nucleotide or bound ADP. Changes in flexibility are apparently concomitant with the force generating step. Bending relaxation times extracted the electric birefringence curves indicate that flexing is occurring at the S1-S2 junction. Coupled with our previous experiments, a previously unsuspected tight coupling of the HMM-LMM and the S1-S2 junction conformations probably exists.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 11500-01 ODIR

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DNA Repair and Replication in *Xenopus*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Eric Ackerman	Special Expert	OD, NIDDK
Others:	Naoko Oda	Visiting Fellow	OD, NIDDK
	Alexander Spoonde	Visiting Fellow	OD, NIDDK
	Jing Zhang	IRTA	OD, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Office of Director

SECTION

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the extensive laboratory renovations following our move to Bldg. 5, there was a complete change in laboratory personnel. For most of the year, only one visiting fellow was present. These events temporarily slowed our progress towards understanding the molecular biology of vertebrate DNA repair. During early development *Xenopus* replicates its DNA nearly as fast as *E. coli* in log phase. We demonstrated that oocytes are an excellent source of both DNA repair activity and single-strand to double-strand replication activity. Pyrimidine dimer and (6,4) photoproduct repair was shown by microinjecting UV-irradiated plasmid DNA into oocytes or adding damaged DNA to an extremely efficient extract derived from oocytes. We have also studied DNA repair for alkylated and chemically modified DNA as well as DNA replication with our repair extract. One of the advantages of the Xenopus system is that we can study repair/replication reactions both in living cells by microinjection and in extremely efficient extracts. Unlike other extracts that repair <2% of the input damaged DNA, our extracts repair all of the damaged substrate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 13001-21 MRB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mathematical formulations and analysis relevant to experimental neurophysiology.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. Rall	Senior Research Physicist	MRB, NIDDK
Others:	I. Segev	Fogarty Visiting Scientist (Summer)	MRB, NIDDK

COOPERATING UNITS (if any)

Dept. of Neuroscience, Hebrew Univ. of Jerusalem

LAB/BRANCH Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.2	OTHER: .3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

RESEARCH AREA. Basic neuroscience involving structure/function relations for neuronal dendritic branching, dendritic spines, and synapses (also neuron populations, with cortical symmetry), and for such functions as synaptic transmission, amplification and dendro-dendritic interactions in the context of spatio-temporal input patterns, logical processing of input, and neural plasticity, as in conditioning and learning.

RATIONALE. Combine experimental data from neuroanatomy and from electrophysiology with biophysical models of nerve membrane (passive, synaptic and excitable) into a comprehensive theory which can lead to new insights and to testable theoretical predictions (leading to the design of better experiments). To do this, we must create, explore and test mathematical and computational models with different degrees of complexity.

METHODOLOGY. Our methods include both analytical solutions and computational solutions of boundary value problems (for partial differential equations) in the traditions of classical physics. They include also the formulation and solutions of problems in terms of systems of ordinary differential equations; when this is done explicitly for a compartmental model of a neuron, it is possible to accommodate a remarkable variety of dendritic branching patterns and non-uniform distributions of membrane properties and of synaptic inputs.

RESULTS. Perspective, results and references can be found in a recent review and in chapters in several recent books: see the Oct 1992 special issue of Physiological Reviews, 72: S159-S186; see also "Single Neuron Computation: (T. McKenna, J. Davis and S.F. Zornetzer, eds) Academic Press, 1992, and "Computational Neuroscience" (E.L. Schwartz, ed.) MIT Press, 1990.

U.S. GOVERNMENT PRINTING OFFICE: 1981 0-364-633

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 13002-22 MRB

PERIOD COVERED
October 1, 1993 to September 30, 1994.

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mathematical Description of Substrate Transport to Capillary-Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Jose Gonzalez- Fernandez   Research Mathematician   MRB,NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, Betehsda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 13004-21 MRB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mathematical Description of Cellular Neuroelectric Signal Transmission

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	John Rinzel	Chief	MRB, NIDDK
Others:	Yue-Xian Li	Visiting Fellow	MRB, NIDDK
	David Golomb	Visiting Fellow	MRB, NIDDK
	Paul Pinsky	Pre-doc IRTA	MRB, NIDDK
	Victoria Booth	IRTA Fellow	MRB, NIDDK
	Richard Bertram	IRTA Fellow	MRB, NIDDK
	Tim Kiemel	Special Volunteer	MRB, NIDDK

COOPERATING UNITS (if any)

S Stojilkovic (ERRB, NICHD), JE Keizer (Univ Calif, Davis), X-J Wang (Univ Pittsburgh), Y Manor, I Segev, Y Yarom (Hebrew Univ), G Shepherd (Yale Univ)

LAB/BRANCH Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS: 4.75	PROFESSIONAL: 4.25	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project continues to focus on the formulation, analysis, and biophysical interpretation of mathematical models which describe various aspects of electrical activity of neurons and other cell types. Among the topics of current interest are: (i) integration of synaptic input delivered to the soma and dendritic branches of a neuron; (ii) propagation of action potentials along axons; (iii) stimulus-response and threshold properties for repetitive-firing of action potentials; (iv) complex bursting patterns of membrane potential oscillations which arise through endogeneous membrane properties and/or intercellular coupling.

Because qualitatively related mathematical or biophysical problems may arise in other context, e.g., chemical and biochemical oscillations, or e.g. excitation-secretion coupling, this project may consider models from such applications.

Mathematical models of these phenomena involve systems of linear and nonlinear ordinary differential equations and parabolic partial differential equations. Solutions and their mathematical stability are determined by analytical and numerical methods drawn from both classical and modern applied mathematics. These methods may include finite difference or finite element numerical integration, bifurcation theory, perturbation methods, and nonlinear dynamical systems theory. One goal of this project is to expose the qualitative mathematical structure for classes of models by exploiting simple, yet physiologically reasonable, equations.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 13020-05 MRB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Electrical and Chemical Oscillations in Coupled Cell Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arthur Sherman	Senior Staff Fellow	MRB, NIDDK
Others:	Richard Bertram	IRTA Fellow	MRB, NIDDK
	Paul Smolen	NRC Fellow	MRB, NIDDK
	Michael Mascagni	Guest Worker	MRB, NIDDK
	Manish Butte	Summer IRTA	MRB, NIDDK
	Tim Kiemel	Guest Worker	MRB, NIDDK

COOPERATING UNITS *(if any)*
JE Keizer (Univ Calif, Davis), C Stokes, L Xu (Dept of Chem Eng, Univ of Houston), B Soria (Dept de Fisiologia, Universidad de Alicante, Alicante, Spain)

LAB/BRANCH Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2.2	PROFESSIONAL: .1	OTHER: 2.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We use mathematical models to study the mechanisms of oscillatory electrical activity arising from ion channels in cell membranes and modulated by intracellular chemical processes. We are interested in both the behavior of single cells and the ways in which cells communicate and modify each other's behavior.

Our main application has been to the biophysical basis of insulin secretion in pancreatic beta-cells. We have examined bursting oscillations in membrane potential and the role of electrical coupling between cells in the islet of Langerhans. Long term goals are to understand how the membrane dynamics interact with intracellular events to regulate secretion and to generalize to other secretory cells and neurons.

Our primary tool is the numerical solution of ordinary and partial differential equations. We use analytical, geometrical, graphical, and numerical techniques from the mathematical theory of dynamical systems to help construct and interpret the models. Perturbation techniques are used to get analytical results in special cases.

We study both detailed biophysical models and simplified models which are more amenable to analysis. Such an approach aids the isolation of the essential or minimal mechanisms underlying phenomena, the search for general principles, and the application of concepts and analogies from other fields.

We see a role for our group as intermediaries between the mathematical and biological disciplines. This includes disseminating the insights of mathematical work to biologists in accessible language and alerting mathematicians and other theoreticians to new and challenging problems arising from biological issues.

U.S. GOVERNMENT PRINTING OFFICE 1991

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 15100-24 LCDB

PERIOD COVERED
October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Protein Nucleic Acid Interactions: Chromatin Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert T. Simpson	Laboratory Chief	LCDB, NIDDK
Others:	Michael P. Kladde	Staff Fellow	LCDB, NIDDK
	Michael Murphy	IRTA Fellow	LCDB, NIDDK
	Hugh-George Patterton	Visiting Fellow	LCDB, NIDDK
	Christopher Szent-Gyorgyi	Senior Staff Fellow	LCDB, NIDDK
	Yuko Tsukagoshi	Visiting Fellow	LCDB, NIDDK

COOPERATING UNITS *(if any)*
T. Richmond, ETH, Zurich, Switzerland (foreign)

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Developmental Biochemistry Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	5.5	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of chromatin structure in modulating the functions of DNA in transcription, replication, recombination and repair is becoming increasingly apparent. We have shown that the yeast alpha2 repressor positions a nucleosome adjacent to its operator. This organized chromatin structure is propagated; at least four additional nucleosomes are positioned with base pair precision. Mutations in yeast genes known to be necessary for repression by alpha2 also lead to perturbation of the organized chromatin structure. Studies moving the transcription initiation site relative to the alpha2 operator demonstrate that repression is involved with chromatin structure above the level of an individual nucleosome. The entire STE6 gene is packaged in organized nucleosomes; the end of the 4 kbp domain is at the 3' end of the structural gene. The domain appears to exist as repeated close packed dimers interspersed by long (42 bp) linkers. This organization has potentially interesting implications for formation of an unique chromatin structure.

Studies defining chromatin features which preclude access of in vivo expressed dam methylase have been completed. Using a methylase which also recognizes GATC but modifies C instead of A, we have adapted a chemical method which gives a positive signal for methylation, facilitating analyses. The Hha methylase which modifies CpG sequences has been expressed in yeast; this offers a modification site every 30-50 bp, on average, allowing chromatin analyses without introduction of restriction sites.

Haploid specific genes are repressed in yeast diploids by an a1/alpha2 heterodimer. Three consensus binding sites and two near-sites are upstream of the SST2 gene, as is an organized chromatin structure. To determine which site(s) were important in repression, we disrupted each of the a1/alpha2 binding sites, singly and in combination. Surprisingly, even with all five sites mutated, a reporter gene remained repressible in diploid cells. The cis-acting elements involved in control of a meiosis specific heat shock gene have been defined. In addition to the heat shock element, an upstream repressor sequence and a sequence which flanks it and modulates basal expression are critical for proper repression and induction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 15102-34 LCDB

PERIOD COVERED
October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of a ribonuclease and its inhibitor from Bacillus amyloliquefaciens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert W. Hartley	Research Physicist	LCDB, NIDDK
	Milan Jucovic	Visiting Fellow	LCDB, NIDDK
	Yelena Chernokalskaya	Visiting Associate	LCDB, NIDDK

COOPERATING UNITS *(if any)*
Dr. Yves Mauguen, Centre Pharmaceutique, Univ de Paris-Sud; Dr. Josef Sevcik, Inst of Molecular Biology, Slovak Acad Sci, Bratislava; Dr. Peter Privalov, Dept. of Biology, The Johns Hopkins U., Baltimore, MD; Dr. Inna Leschinskaya, Kazan University, Kazan, Russia.

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Developmental Chemistry Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.3	2.3	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two proteins, barnase, the extracellular ribonuclease of *Bacillus amyloliquefaciens*, and barstar, its intracellular inhibitor, are used as a model system for the study of protein folding and protein-protein interactions. Barnase is one of an homologous group of ribonucleases occurring in both prokaryotes and eukaryotes. Recombinant DNA techniques are being applied with three major aims: (1) to facilitate production of wild type and mutant proteins; (2) to examine the structural and control sequences of the genes; and (3) to make specific changes in the sequences to test theories of folding and to probe the barnase-barstar interaction. Both proteins can now be obtained from recombinant genes in *E. coli*, where expression of barstar counters the lethal effect of barnase expression. The structures of both proteins and their complex are known. One or more mutations of all of the barstar residues in direct contact with barnase in the complex, as well as several such for barnase, have been prepared and preliminary binding studies have been done for several combinations. Determination of the Gibbs free energy for all combinations of such mutants in complex will isolate the energy contributions of different portions of the interface. Barstar(C42,80A), which binds barnase almost as well as the wild type, is being used for much of this work. A system has been devised to select, *in vivo*, barstar mutants with improved binding to barnase mutants (e.g. H102K) which are poorly inhibited by wild type barstar. Several such barstar mutants have been found, with the strongest suppressors of barnase (H102K) all including the mutation Y30W. As the side chain of this tyrosine is buried within barstar, this suggests that the improved binding involves some structural rearrangement of surrounding residues. *In vitro* studies confirm that *in vivo* behavior correlates with strength of barnase-barstar binding.

We have found that, for at least two different *Streptomyces* ribonucleases (barnase homologs), barstar is a good enough inhibitor to provide *in vivo* protection and thus allow the production of these enzymes in *E. coli*. *In vitro* binding studies and co-crystallization with barstar for structural studies are planned.

Work elsewhere, in which the barnase gene becomes a tissue-specific killer when attached to eukaryotic promoters, has aroused considerable interest in its use in developmental studies and as the key to a variety of anti-viral strategies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15200- 34LCDB

PERIOD COVERED
October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on Folic Acid (Dihydrofolate Reductase) and Vitamin A (Beta-Carotene)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Bernard T. Kaufman	Section Chief	LCDB, NIDDK
Others:	John G. Bieri	Scientist Emeritus	LCDB, NIDDK

COOPERATING UNITS *(if any)*
Dr. J.C. Smith, U.S. Department of Agriculture, Beltsville, MD; Dr. James Freisheim, Medical College of Ohio; Dr. Michele Metigue, University of California at San Diego.

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Nutritional Biochemistry Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1993 - 1994

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 15401-22 LCDB

PERIOD COVERED
October 1, 1993, to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Synthesis and Transport of Lipoprotein and Hepatic Lipases in Cells and Tissues

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robert O. Scow	Chief, Endocrinology Section	LCDB, NIDDK
Others:	Charles J. Schultz	IRTA Fellow	LCDB, NIDDK
	E. Joan Blanchette-Mackie	Research Biologist	LCDB, NIDDK
	Albert E. Spaeth	Chemist	LCDB, NIDDK

COOPERATING UNITS (if any)
Drs. C. C. Hedrick and L. W. Castellani, Dept. of Med., UCLA; Dr. J.-W. Park, Dept. of Biochem., Chonbuk Natl. Univ. Med. School, Chonju, Chonbuk, Republic of Korea; Dr. T. Takahashi, Dept. of Oral Anatomy, Kanagawa Dental College, Yokosuka, Kanagawa, Japan

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lipoprotein lipase (LPL) is required for uptake of plasma lipoprotein triacylglycerol (TG) by tissues. It is synthesized in parenchymal cells and transferred and attached by proteoheparan sulfate to capillaries, where it acts. Active LPL is a noncovalent homodimer of N-linked glycosylated subunits. Active dimeric LPL has higher affinity for heparin than inactive monomeric LPL. Sucrose gradient centrifugation and heparin-Sepharose chromatography showed that most LPL dimers in normal brown adipocytes were active and had high heparin-affinity (HA). When Golgi mannosidase I was blocked with DMM (1-deoxymannojirimycin), only 60% of the dimers were active and had high HA, and the rest were inactive and had low HA. BFA (brefeldin A), which blocked LPL transport from ER (endoplasmic reticulum) and relocated Golgi components to ER, increased to 100% the proportion of LPL dimers in DMM cells that were active and had high HA. Blockade of glucosidase I activity in ER with CST (castanospermine) resulted in synthesis of inactive LPL which had low HA and was retained in ER primarily as higher oligomers. CST cells treated with BFA contained LPL dimers, two-thirds of which were active and had high HA. The findings show that LPL can be present in two forms of dimers in cells. That LPL maturation occurred in ER of cells in which Golgi enzymes were relocated to ER by BFA suggests that processing by Golgi components is necessary for dimerization, high HA and activity of LPL. Our findings suggest that LPL subunits in active dimers are aligned so that heparin binding sites are on the back side and active site regions on the front side of the dimer, allowing active site lids to open freely upon interfacial activation, and both active site regions to interact with the surface of TG-rich lipoproteins.

Combined lipase deficient (*cld/cld*) mice have functional deficiencies of both LPL and hepatic lipase. The adrenal glands of *cld/cld* mice are normal in size, but they have no hepatic lipase activity (required for uptake of plasma CE by adrenals) and 93% less cholesteryl ester (CE) than normal. Plasma concentration of HDL-CE, the primary source of cholesterol for adrenals, is also very low (10% normal) in *cld/cld* mice. These findings indicate that *cld/cld* mice probably die of adrenal insufficiency, secondary to impaired delivery of cholesterol to the adrenals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15404-10 LCDB

PERIOD COVERED

October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ultrastructural Immunocytochemistry of Lipid Metabolism in Cells and Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Joan Blanchette-Mackie	Research Biologist	LCDB, NIDDK
Others:	Robert O. Scow	Chief, Endocrinology Sect.	LCDB, NIDDK
	Nancy K. Dwyer	Biologist	LCDB, NIDDK
	Robert A. Coxey	IRTA, Post-Doctoral Fellow	LCDB, NIDDK
	Therese Barber	IRTA, Pre-Doctoral Fellow	LCDB, NIDDK

COOPERATING UNITS *(if any)*

Dr. Peter Pentchev, Dev Metab Neurol Branch, NINCDS, NIH; Dr. Howard Kruth, Lab Exptl Ather, NHLBI, NIH; Dr. Constantine Londos, Lab Cell Develop Biol, NIDDK, NIH; Dr. Richard E. Pagano, Carnegie Institution of Washington, Baltimore, MD.

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A reversible, cellular cholesterol lipidosis was studied to probe the relationship between the exit of cholesterol from lysosomes and the functional integrity of the Golgi. Although this progesterone-induced accumulation of cholesterol in lysosomes during LDL-uptake is extensive, it did not block enrichment of membranes of trans Golgi cisternae with cholesterol. Thus, progesterone and the Niemann Pick Type-C (NP-C) mutation both induce an accumulation of cholesterol in lysosomes which is linked to a more distal block in cholesterol trafficking at the trans Golgi cisternae. Endoplasmic reticulum is an additional intracellular site at which the processing and transport of cholesterol can be disrupted. During uptake of LDL, inhibition of acyl-CoA:cholesterol acyltransferase (ACAT) with 58035 induced an extensive accumulation of unesterified cholesterol in rough endoplasmic reticulum. There was no visible sequestration of cholesterol in lysosomes even though the amount of unesterified cholesterol in cells treated with the ACAT inhibitor reached levels seen in both NP-C and progesterone-treated cells. Although 58035 blocks ACAT, the drug enhances other homeostatic responses whereas, the progesterone induced block and NP-C mutation inhibit all homeostatic responses. These differences in potency of cholesterol regulation of cell homeostasis can now be related to organelle site of cholesterol accumulation. Cholesterol transfer to endoplasmic reticulum may be necessary to elicit these cellular responses.

A similar lipase hydrolyses lipid stores in adipocytes and steroidogenic cells (hormone sensitive lipase, HSL). Immunocytochemical studies, showed HSL was present on the surface monolayer of most triacylglycerol containing lipid droplets in adipocytes stimulated with isoproterenol, whereas little HSL was associated with the surface of lipid droplets in unstimulated adipocytes. Thus, hormonal stimulation of adipocytes increases access of intracellular HSL to its triacylglycerol substrate. A second pool of HSL was found in mitochondria of both adipocytes and steroidogenic cells. The location of HSL in mitochondria and their close relationship with the surface of intracellular lipid droplets would facilitate transfer of lipolytic products, cholesterol and fatty acids, to mitochondria for further metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15500-33 LCDB

PERIOD COVERED

October 1, 1993, to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Large-Scale Production and Purification of Compounds with Biological Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Joseph Shiloach	Research Chemist	LCDB, NIDDK
Others:	Jeanne B. Kaufman	Biologist	LCDB, NIDDK
	Amos M. Tsai	Pre-IRTA Fellow	LCDB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Biotechnology Unit, Office of the Chief

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Biotechnology Unit is responsible for:

a. Large-scale production of procaryotes (bacteria), and eucaryotes (mammalian cells, insect cells).

b. Large-scale recovery and purification of biologically active compounds (proteins, polysaccharides, etc.) from various sources.

c. Process and method development associated with: (1) bacterial growth; (2) eucaryotic cell growth; and (3) purification of biologically active compounds, especially proteins. The process development work is conducted to develop a procedure for large-scale preparation and purification of material suitable for clinical trials.

d. Research and development work not necessarily linked to a current process development project, but work that has long-term implications for fermentation processes and protein purification.

During the last 12 months, the Unit performed 250 different large-scale preparations.

The unit continues its effort to produce a large amount of proteins needed for various clinical use: S antigen from bovine retina, currently in the second phase of the clinical trials, recombinant pseudomonas aeruginosa exotoxin A (without its toxic function) needed for the preparation of conjugated vaccine, and mutant diphtheria toxin. The facility continues to prepare large quantitites of various human plasma cell lines for preparation and purification of peptides associated with HLA proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15503-13 LCDB

PERIOD COVERED

October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Developmental Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Alan R. Kimmel	Research Chemist	LCDB, NIDDK
Others:	John L. Medabalimi	Senior Staff Fellow	LCDB, NIDDK
	Gail Ginsberg	Biologist	LCDB, NIDDK
	Rachel Gollop	Visiting Fellow	LCDB, NIDDK
	Jasmine Gruia-Gray	Visiting Fellow	LCDB, NIDDK
	Delwood Richardson	Senior Staff Fellow	LCDB, NIDDK
	Kelley Rogers	IRTA Fellow	LCDB, NIDDK
	Peter Balint-Kurti	IRTA Fellow	LCDB, NIDDK

COOPERATING UNITS *(if any)*

Dr. Constantine Londos, Membrane Regulation Section, LCDB, NIDDK

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Molecular Mechanisms of Development Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.5	6.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development of Dictyostelium discoideum requires response to extracellular cAMP signalling that is mediated through a family of G protein-linked surface receptors. We had isolated the four members of the cAMP receptor (CAR) family and now determine the spatial regulation of each. The developmental function of CAR4 has been studied in the most detail. CAR4 is a low affinity cAMP receptor that is maximally expressed in prestalk cells and may be required both to establish and to sense the extracellular cAMP signal during late development. Some prestalk cells of car4-null structures are absent or mis-localized. This phenotype is cell autonomous. In contrast, the aberrant patterning of car4-null prespore cells is non-autonomous. Two putative transcriptional regulators of cell fate in Dictyostelium have been identified. One is associated with a mutation that blocks spore differentiation and shares 65% identity with the Brahma-family of regulatory proteins. The other, rfZIP, has a zinc finger and leucine zipper domain. Mutant cells develop poorly and exhibit both cell autonomous and non-autonomous effects on patterning and differentiation. We have also identified promoter sequences for several genes and putative sequence-specific DNA binding proteins that are essential for cell specific-gene expression or response to a distinct intracellular signalling cascade.

Lipolysis in mammalian adipocytes requires translocation of hormone sensitive lipase from the cytosol to the lipid droplet surface. Perilipins are located at the droplet surface and are potential regulators of lipolysis. We have constructed cell lines that accumulate lipid droplets without associated perilipin. Analysis of these cells will indicate a potential requirement for perilipin in the activation or inhibition of lipolysis. We have also identified two additional perilipin species and their differential expression in adipogenic and steroidogenic cells. They potentially have distinct regulatory functions in these different cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15505-17 LCDB

PERIOD COVERED

October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Adipocyte Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Constantine Londos	Research Chemist	LCDB, NIDDK
Others:	Janice L. Theodorakis	IRTA Fellow	LCDB, NIDDK
	Christina Rondinone	Visiting Fellow	LCDB, NIDDK
	Daniel M. Levin	Special Volunteer	LCDB, NIDDK
	Diane L. Servetnick	Biologist	LCDB, NIDDK
	Dawn L. Brasaemle	Senior Staff Fellow	LCDB, NIDDK
	Charles J. Schultz	IRTA Fellow	LCDB, NIDDK

COOPERATING UNITS *(if any)*

A.R. Kimmel, J. Blanchette-Mackie, J. Gruia-Gray, LCDB, NIDDK; J. Wolff, LBP, NIDDK; R.P. Nordan, DCT, NCI; N. Edens, University of Maryland

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Membrane Regulation Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Triacylglycerols in adipocyte lipid storage droplets are the primary energy reserves in animals, and fatty acids are marshaled by a cAMP-dependent protein kinase (PKA)-mediated process. PKA phosphorylates both hormone-sensitive lipase (HSL), the rate-limiting enzyme of lipolysis, and perilipins, proteins that coat the surface of lipid droplets. By an analogous system, steroid hormone synthesis is initiated by the hydrolysis of cholesteryl esters (CE). We find that adrenal cortical and Leydig cells express perilipins, which are located exclusively at the surface of CE droplets. Murine adipocytes and steroidogenic cells express 4 perilipins (A, B, C, D); the A species is the most abundant in all cells, whereas the C species is relatively abundant in steroidogenic cells but rare in adipocytes. Given the parallels between the modes of lipid hydrolysis in adipocytes and steroidogenic cells, the finding of perilipins in both types of cells supports the idea that perilipins play a role in lipid metabolism.

Experimental evidence supports our proposal that translocation of HSL to lipid droplets is a critical event in lipolysis. We find by immunofluorescence that HSL is distributed diffusely in the cytoplasm of unstimulated adipocytes; upon stimulation, HSL migrates rapidly to lipid droplets. Test systems have been developed to further dissect the lipolytic reaction and to examine the targeting of perilipins to lipid droplets. These include 3T3-L1 adipoblasts stably transfected with both sense and antisense perilipin cDNA constructs as well as adrenal cortical cells transfected with cDNA constructs bearing epitope tags at both N- and C-terminal regions of perilipin A. The products of all such constructs localize to lipid droplets. Interestingly, adipoblasts containing the sense perilipin constructs fail to differentiate, but contain perilipin associated, paradoxically, with TG droplets suggesting a role for perilipins in lipid packaging.

Cytokines that have been implicated in disease-associated cachexia are TNF, IL-6, and LIF. We have found that these agents increase the tyrosine phosphorylation and activity of MAP kinases, but that IL-6 and LIF also increases the tyrosine phosphorylation of several cytosolic proteins. Two such proteins of 90/92 kDa appear to be STAT3, transcription factors that translocate to the nucleus upon phosphorylation. These events may underlie actions that contribute to cachexia, such as reduced expression of lipoprotein lipase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15506-10 LCDB

PERIOD COVERED

October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Control of Gene Expression in Mammalian Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jurrien Dean	Section Chief	LCDB, NIDDK
Others:	Li-Fang Liang	Staff Fellow	LCDB, NIDDK
	Mary Familari	Visiting Associate	LCDB, NIDDK
	Ann Ginsberg	Staff Fellow	LCDB, NIDDK
	Robert McIsaac	IRTA Fellow	LCDB, NIDDK
	Roxanne Chan	Visiting Fellow	LCDB, NIDDK
	Olga Epifano	Special Volunteer	LCDB, NIDDK
	Gail Osawa	Chemist, GS-11	LCDB, NIDDK

COOPERATING UNITS *(if any)*

Zhi-Bin Tong and Larry Nelson, DEB, NICHD; Malcolm Moos, DBB, CBER

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Mammalian Developmental Biology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.0	7.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mouse ovary serves as a paradigm for investigating the developmental biology of mammalian gonadogenesis, oogenesis and fertilization.

Gonadogenesis: Mouse gestation occurs over 20 days. Germ cells, first detected in the developing embryo 7.5 days post coitus (dpc), migrate from the allantois to the genital ridge by 12.5 dpc. In XX females, the primitive gonad then differentiates into an ovary. Poly(A$^+$) was purified from female and male genital ridges isolated 12-13 dpc. cDNA libraries have been made for each sex and subtractive cloning techniques are being used to identify female-specific gene products involved in early sex determination and gonadogenesis.

Oogenesis: The expression of the zona pellucida, an ovary-specific extracellular matrix composed of three glycoproteins (ZP1, ZP2, ZP3), serves as a marker of oocyte growth and differentiation in the adult female. A 12bp DNA sequence 200bp upstream of the start of transcription of the *Zp*-2 and *Zp*-3 genes is necessary and sufficient for zona promoter activation. Using yeast genetics, efforts are underway to clone ZAP-1, the putative transcription factor that binds to this DNA element. These studies will provide molecular details of mechanisms involved in the coordinate, oocyte-specific expression of the zona genes. ZAP-1 will additionally provide a marker of early oocyte growth and differentiation.

Fertilization: The three zona proteins are secreted and form an extracellular matrix that mediates the relatively species-specific events of fertilization. Using microinjection techniques to degrade endogenous zona transcripts and to introduce synthetic zona mRNAs, we are assessing protein-protein interactions in the assemblage of the zona matrix. To investigate the molecular biology of sperm-egg interactions mediated by the zona, we are using recombinant DNA techniques to express mouse and human zona proteins and to create mutant zona proteins, including mouse/human chimeric proteins. These will be tested for their ability to competitively inhibit fertilization, enabling establishment of structure-function correlations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 15508-06 LCDB

PERIOD COVERED
October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chromatin Structure in Regulation of Mammalian Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ann Dean	Research Chemist	LCDB, NIDDK
Others:	Qi-Hui Gong	Visiting Scientist	LCDB, NIDDK

COOPERATING UNITS *(if any)*
Genevieve Almouzni, LME, NICHD

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Developmental Biochemistry Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The members of the beta-globin family of genes are expressed sequentially during human development. The epsilon-globin gene is the first to be expressed, is actively transcribed until the sixth week of development, and is inactive later in development. Expression of the beta-like-globin genes is under the control of the beta-globin locus control region (LCR), encompassing erythroid specific DNase I hypersensitive sites (HS) 5-18 kilobases upstream of the epsilon-globin gene.

The LCR has long range effects on the chromatin structure of the beta-globin locus, and it activates transcription of the different globin gene promoters as the genes are expressed during development. Using *in vitro* protein-DNA binding studies, clustered point mutations, and transient expression assays with a reporter gene, we have identified transcription factors that can be expected to participate, *in vivo*, in mediating the interaction of the LCR with the epsilon-globin gene promoter. These include the ubiquitous transcription factor Sp1 (or a related family member) and the erythroid transcription factors GATA-1 and NF-E2.

To study the promoter-LCR interaction in the context of chromatin *in vivo* we have constructed a minichromosomal vector containing a marked epsilon-globin gene. Expression of the minichromosomal epsilon-globin gene in K562 human erythroid cells is dependent on the presence of the beta-globin LCR *in cis*. Regulated expression of the epsilon-globin gene on the minichromosomes offers a means to study at high resolution the effects of the LCR on the chromatin structure of the epsilon-globin gene, and the possibility to dissect the promoter-enhancer interaction of an activated gene assembled *in vivo* into chromatin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 15509-03 LCDB

PERIOD COVERED
October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Polyprotein Processing in Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John L. Medabalimi	Senior Staff Fellow	LCDB, NIDDK
	Ewald M. Wondrak	Visiting Associate	LCDB, NIDDK
	Alan R. Kimmel	Research Chemist	LCDB, NIDDK

COOPERATING UNITS *(if any)*
Drs. N.T. Nashed, M.T. Haber, and D.M. Jerina, Section on Oxidative Mechanisms, LBC, NIDDK; Drs. K. Sakaguchi and E. Appela, LCB, NCI

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Molecular Mechanisms of Development Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have investigated the mechanism of autoprocessing of the human immunodeficiency virus, type 1, protease from a polyprotein that contains flanking sequences at the protease's termini [MBP-12AA-PR-19AA]. The dimeric MBP-12AA-PR-19AA protein has an active site similar to that of the mature enzyme and can bind inhibitors and substrates, although its enzymatic activity is much lower than that of the mature protease. The N-terminal cleavage is an intramolecular reaction that occurs much faster than the C-terminal cleavage and leads to the accumulation of the intermediate protein, PR-19AA (13.2 kDa). The 13.2 kDa protein was purified to near homogeneity by chromatography on a size exclusion column under denaturing conditions. A comparison of inhibition constants, kinetic parameters for the hydrolysis of a peptide substrate, and pH rate profiles indicate that the 13.2 kDa protein has an active site that is similar to that of the mature protease. The major difference between the 13.2 kDa protein and the mature enzyme is in their susceptibility to urea denaturation. Initial rates of the conversion of the 13.2 kDa protein to the mature protease is dependent on the square of the protein concentrations indicating that the C-terminal sequence is cleaved via an intermolecular mechanism in the autoprocessing reaction. To compare the interface contacts of the homodimeric protease fusion protein with that of the mature protease we have expressed and purified an inactive fusion protein. This fusion protein inhibits the mature wild-type protease through heterodimer formation.

In retroviral assembly, RNA selection and packaging involves the two cysteine arrays of the nucleocapsid (NC) protein which form zinc fingers. We demonstrate specific cleavage within the first finger of the HIV-1 NC protein by the viral protease that is dependent upon removal of zinc ions. The reaction is time dependent and is completely inhibited by 1 mM pepstatin A, a specific inhibitor of aspartic acid proteases. Also, 3-nitrosobenzamide, a compound shown to have antiviral properties, and cupric ions, that release zinc through oxidation of the cysteine residues of the finger, render the NC protein resistant to cleavage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 17001-28 LBM

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Role of Carbohydrate Moiety of Glycoproteins in Cellular Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. Ashwell	Institute Scholar	LBM, NIDDK
Others:	W. Berlin	IRTA	LBM,
NIDDK	O. Gabriel	Parttime Special Volunteer	LBM, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 2.0	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The work carried out this year may be divided into four separate projects. Of these, two represent a continuation of studies begun earlier on the presence and identification of intranuclear glycoproteins. Considerable progress has been made in identifying the nuclear HMG proteins as being responsible for the aberrant hydrazide labeling reaction and points to the probability of a role for specific nuclear protein crosslinking. Further evidence has also been obtained to confirm the presence and identification of intranuclear glycogen, a project which has taken on added significance in the light of recent reports documenting an unsuspected role for glycogen as an essential requirement for nuclei formation around naked DNA in an in vitro system. A new project undertaken this year has resulted in the development of a specific assay for polysialic acid, a critical component of neural cell adhesion molecules (N-CAMs). Finally, a series of genetic studies have been initiated in an attempt to isolate the gene encoding MHL-1 as a prelude to knock-out experiments designed to provide insight into the unique role of the hepatic asialoglycoprotein receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 17002-24 LBM

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Enzymatic Basis of Detoxication

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W.B. Jakoby	Chief, LBM	LBM, NIDDK
Others:	D. Marshall	Visiting Fellow	LBM, NIDDK
	X. Xie	IRTA	LBM, NIDDK

COOPERATING UNITS (if any)
Peter McPhie, Laboratory of Biochemical Pharmacology, NIDDK

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Enzymes and Cellular Biochemistry Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

As part of a program in which the enzymes that participate in the process of detoxication are being examined as to their catalytic mechanism, attention is being concentrated on a sulfotransferase (aryl sulfotransferase IV). The enzyme catalyzes the transfer of the sulfuryl group from 3-phosphoadenosine 5-phosphosulfate (PAPS) to a very broad range of phenols; the products are 3-phosphoadenosine 5-phosphate (PAP) and the corresponding phenol sulfate. Thereby, lipophilic and toxic phenols are converted to more water soluble compounds that are readily excreted. One of the characteristics of enzymes that participate in detoxication is just this large variety of lipophilic xenobiotics that serve as substrates.

This laboratory has cloned the sulfotransferase from rat liver mRNA and can express it in very large quantities from Escherichia coli. In purifying the recombinant enzyme, however, two distinct forms of the sulfotransferase were physically separable. Labeled as forms A and B, both catalyzed the reverse reaction, i.e. the formation of free phenol from the phenol sulfate and PAP, but only form B could catalyze the physiological reaction. Further investigation revealed that form A had PAP bound to it so tightly that it would not allow the physiological donor, PAPS, to bind to the enzyme. The question under investigation is the nature of the tight binding of PAP to form A which presently appears to result from protein refolding after migration of a disulfide bond.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 17003-27 LBM

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Polysaccharides in Morphogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Cabib	Senior Research Chemist	LBM, NIDDK
Others:	J.A. Shaw	Staff Fellow	LBM, NIDDK
	R. Ford	IRTA	LBM, NIDDK
	W.-J. Choi	Visiting Fellow	LBM, NIDDK
	Roman Kollar	Special Volunteer	LBM, NIDDK
	Emanuela Lacanà	Visiting Fellow	LBM, NIDDK

COOPERATING UNITS *(if any)*

LCB, NHLBI (Blair Bowers); Departmento de Microbiologia, University of Salamanca, Spain (Angel Duran); Slovak Academy of Sciences, Institute of Chemistry, Bratislava, Czechoslovakia (Vladimir Farkas); CRADA, American Cyanamid, Princeton, NJ

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Morphogenesis

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
4.5	4.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new study of chitin synthetase 3 from yeast has led to the conclusion that under certain conditions this enzyme, like chitin synthetases 1 and 2, behaves as a zymogen. Of the three genes necessary for chitin synthetase 3, only *CAL1/CSD2* and *CAL3* are required for the zymogenic activity, whereas the product of *CAL2* appears to act as an activator.

A method for the simultaneous determination of the three yeast synthetases in a mixture has been developed.

The GTPase activity of a GTP-binding protein that regulates ß(1->3)glucan synthetase has been found to be modulated by an apparent GTPase activating protein (GAP) that may link cell wall synthesis with cell cycle controls.

Identification of the linkage between chitin and ß(1->3)glucan in the yeast cell wall has been completed.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 17008-11 LBM

PERIOD COVERED October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Role of the Nuclear Envelope in Intracellular Protein Sorting

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.A. Hanover	Research Chemist	LBM, NIDDK
Others:	S. Bailer	Visiting Fellow	LBM, NIDDK
	W. Lubas	Research Associate	LBM, NIDDK
	M. Miller	IRTA	LBM, NIDDK
	T. Sweitzer	IRTA	LBM, NIDDK

COOPERATING UNITS (if any)

Laboratory of Biochemistry and Metabolism

LAB/BRANCH

Section on Cell Biochemistry

SECTION

NIDDK, NIH, Bethesda, Maryland 20892

INSTITUTE AND LOCATION

TOTAL MAN-YEARS: 4.5	PROFESSIONAL: 4.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The extracellular signals controlling gene expression must ultimately be communicated to the nucleus. Nuclear transport plays a key role in regulating the levels and activities of transcription factors, nuclear kinases, steroid hormone receptors and replication factors. The nuclear pore complex (NPC) facilitates the movement of mRNA and proteins across the nuclear envelope. Previously, we described a family of phosphorylated glycoproteins which are components of the NPC. The major nuclear pore protein, p62, was cloned and expressed as a recombinant protein in *E. coli* and baculovirus. Sites of phosphorylation and glycosylation in p62 were mapped to the central Ser/Thr rich region of the molecule. Interestingly, changes in the degree of phosphorylation and glycosylation oscillated with the cell cycle. Phosphorylation reached a maximum during M phase; glycosylation peaked during S phase. This reciprocal regulation of phosphorylation and glycosylation may be associated with assembly and disassembly of NPC during the cell cycle.

Using digitonin permeabilized cultured cells, we developed means of studying nuclear and nucleolar transport of fluorescent conjugates bearing the appropriate localization signal. Such conjugates concentrated in the nucleus and nucleolus, respectively, when supplied with a cytosolic fraction and ATP. Nuclear transport was strongly activated by addition of Ca^{+2} or GTP to depleted cytosol. IP_3 and calcium chelators inhibited transport; GTP-γ-S also inhibited nuclear transport. The responsiveness of nuclear transport to Ca^{+2} and GTP suggests that nuclear transport may be coordinately regulated with other signal transduction pathways during cellular activation. We have extended these studies using patch-clamp electrophysiology to examine regulated ion flow through the NE of cardiac myocytes. We detected large conductance ion channel activity at the NE resembling that of gap junction channels. Activation of these channels required cytosolic factors. The channels were blocked by GTP-γ-S, wheat germ agglutinin and nuclear pore-specific antibodies. Thus the large conductance nuclear ion channel activity is likely to be intrinsic to nuclear pores.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 17009-09 LBM

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tissue Specific and Hormone Regulated Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. Hennighausen	Research Chemist	LBM, NIDDK
Others:	R.A. McKnight	Staff Fellow	LBM, NIDDK
	K. Heermeier	Visiting Fellow	LBM, NIDDK
	E. Kubiczek	pre-IRTA	LBM, NIDDK
	G. Robinson	Visiting Associate	LBM, NIDDK

COOPERATING UNITS (if any)

Robert Wall (USDA), Priscilla Furth (U. of Maryland, Medical School), Gilbert Smith (NCI), Ed Ginns (NIMH), Peter Gruss (Max-Planck-Institute, Göttingen, Germany), Jeff Pollard (Albert Einstein Medical School), Henryk Lubon (American Red Cross)

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Developmental Biology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Development of the mammary gland during puberty, pregnancy, lactation and involution is controlled by growth modulators which function in a systemic, paracrine and autocrine fashion. We have shown that TGF-ß1 and an endogenous milk protein, the whey acidic protein (WAP), are modulators of mammary development. Temporal deregulated expression of these proteins in mammary epithelial cells of transgenic mice resulted in abrogated mammary development. Furthermore, ectopic synthesis of the WAP under the control of the MMTV-LTR resulted in hyperplasias in the coagulation gland. No growth aberrations were observed in other expressing tissues, such as the salivary gland. This suggests that WAP exerts its growth modulatory effects in a cell-specific manner.

The role of regulatory proteins in tissue formation and differentiation processes can be evaluated using 'gain of function' experiments in transgenic animals. However, the available systems do not permit a temporally controlled activation and inactivation of transgenes. We therefore established a binary system in which transcription of the transgene can be modulated by tetracycline. The reporter transgene (e.g. luciferase, ß-galactosidase or a growth modulator) by itself is inactive because it is under the control of a basal promoter and seven *tet*op sequences. Transcription of the reporter gene can be activated by a fusion protein containing the *tet*R and the activation domain VP16 from the Herpes simplex virus. This fusion protein is expressed under the control of a specific promoter/enhancer. Transcriptional activation can be controlled by tetracycline. The fusion protein binds to the *tet*op only in the absence of tetracycline and stimulates trancription. In the presence of tetracycline the reporter gene is silent. Such a system will be useful in regulating the temporal expression of growth factors and oncoproteins.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 17024-11 LBM

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Inherited Disorders of Lysosomal Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. Myerowitz	Research Chemist	LBM, NIDDK
Others:	J. Tropea	IRTA	LBM, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Inherited Metabolic Disease

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was terminated in 1993. The principal investigator resigned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 18007-15 LBM

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Electrochemical Ion Gradients as a Mechanism of Cellular Message Transmission

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

E.F. Grollman	Medical Officer (Research)	LBM, NIDDK
A. Fanelli	IRTA Fellow	LBM, NIDDK

COOPERATING UNITS *(if any)*

LBM, NIDDK (G. Ashwell, A.R.Robbins); Roswell Park Cancer Inst. (J.YT Lau); Ito Hospital (K. Tahara).

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Enzymes and Cellular Biology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.65	1.40	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The first step in thyroid hormone formation is iodide transport in the thyroid, and is the concern of this project. The project includes studies 1) characterizing iodide transport mechanisms using anion transport inhibitors, 2) transfecting cells with anion transport proteins, and 3) expression cloning of iodide transport activity using cultured thyroid cells that respond to TSH but do not transport iodide. Initial studies focus on the use of specific probes for iodide transport; these probes include stilbene derivatives that interact with anion exchange proteins, and N-substituted anthranilic acid derivatives reported to block non- selective chloride channels in a variety of epithelial cell types. Effects of these compounds on sodium iodide uptake in thyroid cells suggest they may be useful in identifying and isolating iodide-transport proteins. Studies on cells transfected with a cDNA of a non-erythroid cell anion exchanger continue to support prior work that implicates this protein in iodide loss into the thyroid follicle, the site of thyroid hormone formation.

Collaborative studies demonstrated the role of thyrotropin in sialylation of thyroglobulin in cultured rat thyroid cells. These observations have been extended to studies on thyroid tissue from patients with Graves' disease, where thyroglobulin is found to be hypersialylated. This contrasts with thyroglobulin isolated from endemic goiters which is both hypo-iodinated and hypo-glycosylated. The findings continue to support a role for sialylation in thyroglobulin secretion and iodination that is necessary for thyroid hormone formation.

U.S. GOVERNMENT PRINTING OFFICE 1991 0—[illegible]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 18008-28 LBM

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cell Regulation by Hormones, Growth Factors, Autoantibodies, and Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.D. Kohn	Medical Director, USPHS, and Chief, Section on Cell Regulation	LBM, NIDDK
Others:	A. Hidaka	Visiting Fellow (6 mos)	LBM, NIDDK
	H. Shimura	Visiting Fellow	LBM, NIDDK
	Y. Shimura	Visiting Fellow	LBM, NIDDK
	C. Giuliani	Visiting Fellow	LBM, NIDDK
	M. Ohmori	Visiting Fellow	LBM, NIDDK
	M. Saji	Special Volunteer	LBM, NIDDK
	G. Napolitano	Special Volunteer	LBM, NIDDK
	S-I. Taniguchi	Visiting Fellow (5 mos)	LBM, NIDDK
	M. Ohta	Visiting Fellow (4 mos)	LBM, NIDDK
	K. Suzuki	Visiting Fellow (8 mos)	LBM, NIDDK

COOPERATING UNITS *(if any)*

NCI (D. Singer, S. Kimura); U. Texas Galveston (B. Prabhakar); Baylor U., Houston (F. Ledley); U. MD (W.A. Valente); Johns Hopkins Med. Schl., MD (M. Zeiger, M. Levine, N. Rose); U. Naples, Italy (R. DiLauro, E. Avvedimento, S. Alqi, & E. Consiglio); U. Udine, Italy (F.S. Ambesi-Impiombato); U. Milan, Italy (R. Scorza); U. Chieti, Italy (F. Monaco, S. Iacobelli); Tokyo Women's U, Tokyo, Japan (O. Isozaki); U. of Kyoto, Kyoto, Japan (T. Akamizu, S. Kosugi, T. Mori); Weizman Inst. Science, Israel (E. Mozes).

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Cell Regulation

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 9.25	PROFESSIONAL: 8.5	OTHER: .75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This lab has defined structure-function relationships of the thyrotropin receptor (TSHR) and transcriptional regulation of the TSHR with respect to their influence on thyroid growth vs differentiation, signal transduction, gonadotropin receptor regulation of growth vs differentiation, and the action of autoantibodies causing autoimmune thyroid disease. Epitopes for thyroid stimulating and blocking autoantibodies have been defined and related to the immunodominant peptide of the receptor. Cytoplasmic loop links to G-protein interactions and signal transduction mechanisms have been characterized. A minimal TSHR promoter having key properties of the native promoter, i.e. thyroid-specific expression, cAMP autoregulation, and insulin-dependent expression, has been defined and specific elements characterized. Unusual single-strand binding sites and their trans factors have been uncovered and linked to coregulation of TSHR and major histocompatibility (MHC) gene expression, class I and class II. The ability of mutant receptors and TSH crosstalk with gonadotropin receptors to cause gonadal hyperfunction has been described in vitro and in vivo. Sites on the 5'-flanking region of the MHC class I gene, which control its expression by hormones and methimizole (MMI) as well as the influence of interferon on them, have been uncovered and related to the development of thyroid as well as other organ-specific autoimmune diseases, including systemic lupus erythematosus and diabetes. Relationships between transcription factors regulating TSHR and MHC class I promoters have been uncovered and have been shown to be potentially useful to define MMI-derivatives better able to prevent autoimmune disease sequelae and develop donor cells for transplantation or gene therapy.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 18009-15 LBM

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Endocytosis, Secretion and Compartmentalization in Mutant CHO Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. R. Robbins	Research Geneticist	LBM, NIDDK
Others:	C. W. Hall	Research Chemist	LBM, NIDDK
	R. D. Ward	IRTA	LBM, NIDDK

COOPERATING UNITS (if any)
Office of Naval Research, Dept. of Defense (Dr. Constance Oliver)

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It was previously shown that a single amino acid substitution in the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) resulted in increased association of that enzyme with microtubules. This mutant GAPDH was discovered in a CHO cell exhibiting altered endocytosis. It has now been shown that transfection of wild-type CHO cells with GAPDH cDNA containing this mutation results in these same changes in endocytic activity; based on steady-state RNA levels about 15% of the total GAPDH in the stable transfectants contains the amino acid substitution. The principal effect of the mutant GAPDH on endocytosis appears to be increased microtubule-dependent centrifugal movement of late endocytic compartments (late endosomes and lysosomes). The mechanisms underlying the action of this mutant enzyme, as well as of other mutant forms of GAPDH generated *in vitro*, are currently under investigation.

U.S. GOVERNMENT PRINTING OFFICE 1991 O—[illegible]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 18010-07 LBM

PERIOD COVERED October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Role of Intracellular Traffic in HIV Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.A. Hanover	Research Chemist	LBM, NIDDK
Others:	D.W. Frank	IRTA	LBM, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH Laboratory of Biochemistry and Metabolism

SECTION Section on Cell Biochemistry

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transport across the nuclear membrane is a necessary component of the life cycle of the human immunodeficiency virus (HIV). Once in the cytoplasm, HIV RNA is reverse-transcribed into double-stranded DNA which then must enter the nucleus. A growing amount of evidence points to the viral matrix protein of HIV as a mediator of this transport event. Efforts are underway to determine how the matrix protein may be involved in nuclear transport of the DNA-RNA-protein complex. In addition, other retroviral regulatory proteins also enter the nucleus, and viral transcripts are exported out to the cytoplasm. The viral proteins, *rev* and *tat*, have been identified as a key regulators of the transcription and transport of HIV envelope mRNA out of the nucleus. These proteins contain sequences that are necessary and sufficient for targeting to the nucleolus. An *in vitro* system using permeabilized cells has been developed to look at the nucleolar targeting of proteins bearing such targeting signals. This nucleolar localization is blocked by reducing the temperature to 4°C or by omitting ATP. This suggests that transport to the nucleolus is an active process. Recently, it has been demonstrated that a reduction in cellular GTP levels induced by inhibitors of IMP-dehydrogenase reduce the nucleolar accumulation of other nucleolar proteins. However, these inhibitors, which include ribavirin and mycophenolic acid, did not affect nucleolar accumulation of the model protein in permeabilized cells. Moreover, an 100-fold reduction of GTP levels in the *in vitro* assay did not affect nucleolar accumulation of the model protein. Our studies suggest that the loss of a protein with a rapid turnover rate may be responsible for the observed effect of IMP dehydrogenase inhibitors on nucleolar targeting. In other studies, the structure of the nuclear pore has been examined. Nuclei have been reconstituted from *Xenopus laevis* egg extracts with exogenous DNA. The reconstituted nuclei mimic interphase nuclei in many ways and carry out active nuclear transport, and also contain structures resembling nucleoli. The nuclear pore requires glycoprotein components for proper morphology and function.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 18012-10 LBM

PERIOD COVERED
October 1, 1993 to September 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Direct Measurement of Forces between Membranes or Macromolecules

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: V.A. Parsegian	Guest Researcher	LBM, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Enzymes and Cellular Biochemistry Section/Biophysics Unit

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
    ☐ (a1) Minors
    ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 18013-07 LBM

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physics of Ionic Channels and other Proteins with Aqueous Cavities

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	V.A. Parsegian	Guest Researcher	LBM, NIDDK
	S.M. Bezrukov	Visiting Scientist	LBM, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Enzymes and Cellular Biochemistry Section/Biophysics Unit

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been transferred to Z01 DK 11000-01 ODIR.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 18014-10 LBM

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Physical Properties of DNA and DNA-Protein Complexes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: D.C. Rau Research Chemist LBM, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Enzymes and Cellular Biochemistry Section/Biophysics Unit

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

  ☐ (a1) Minors

  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been transferred to Z01 DK 11001-01 ODIR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 18017-01 LBM

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Recombinase-mediated control of gene expression and genome structure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Sauer	Expert	LBM, NIDDK
Others:	B. Bethke	IRTA	LBM, NIDDK
	J. Sztein	Visiting Associate	LBM, NIDDK

COOPERATING UNITS *(if any)*
LBM, NIDDK (G. Ashwell); NICHD (H. Westphal)

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 3	PROFESSIONAL: 3	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Generation of transgenic animal models and production of useful genetically engineered mammalian cell lines is highly dependent on endogenous DNA recombination mechanisms (either homologous recombination or illegitimate recombination) for stable incorporation of exogenous DNA into the genome. We have developed an alternative and complementary recombination methodology based on the prokaryotic site-specific recombinase Cre from phage P1. Expression of Cre recombinase in mammalian cells catalyzes DNA recombination between specific 34 bp *loxP* sites that we have previously engineered into the genome by standard gene transfer methodologies. Because these sites do not exist naturally in the mouse genome, recombination occurs specifically at the sites placed into the genome. By simply choosing how and where such sites are placed on the DNA, Cre recombinase can specifically and precisely delete a preselected DNA segment from the genome. Moreover, because Cre also catalyzes intermolecular recombination, exogenous DNA containing a *loxP* site can be precisely targeted for integration at a *loxP* site previously placed into the genome, thus allowing the facile production of isogenic genetically engineered cell lines.

We have used this methodology to remove unwanted DNA (such as selectable markers, etc.) from the genomes of yeast and transgenic mice. Moreover, we have exploited this recombination system to design a binary transgenic mouse scheme that allows the controlled expression of potentially toxic genes or that can be used to generate a somatic knockout mutation. Combining this binary recombination system with one of the numerous inducible gene expression systems has the potential of allowing the generation of a somatic knockout in a transgenic animal by the simple administration of a chemical compound.

U.S. GOVERNMENT PRINTING OFFICE 1991 O—

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 21019 12

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Hormone and Transmitter Secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Harvey B. Pollard, Chief, LCBG:NIDDK, Others: G. Lee, Ph.D., Res. Chemist; E. Rojas, Ph.D., VS; I. Atwater, Ph.D., Res. Bio; M. Levine, M.D., Sen. Inv., Med. Off.; A. Burns, Ph.D., Res. Bio.; M. Srivastava, Ph.D., SSF; C. McCutchen, Ph.D., Res. Phys.; G. Kuijpers, Ph.D., VA; G. Goping, BM Tech.; M. Adeyemo, Ph.D., SV; F. Vargas, DDS, FDA; K. Dhariwal, Ph.D., SSF; R. Welch, Ph.D., IRTA; Y-H Wang, M.D., IRTA; H. Caohuy, B.S., Biologist; C. Guay-Broder, B.S., Biologist; N. Arispe, Ph.D., VA; Y. Raviv, Ph.D., SV; L. Vergara, M.D., VS; O. Eidelman, Ph.D., SV; E. Flores, MS, SV; R. Vinet, Ph.D., SV; E. Cohen, Ph.D., SV; S. Bar-Noy, Ph.D., SV; M. Glasman, Ph.D., VA; N. Merezhinskaya, Ph.D., VA; P. Raynal, Ph.D., VF; A. Cardenas, Ph.D., SV

COOPERATING UNITS *(if any)*

Jack Cohen, Georgetown University; John Hallenbeck, USUHS

LAB/BRANCH

Laboratory of Cell Biology and Genetics

SECTION

Section on Cell Biology and Biochemistry

INSTITUTE AND LOCATION

NIDDK:NIH, Building 8, Room 403, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	5	-

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Regulation of secretory processes and the mechanisms of external activation and internal control by calcium continue to be the principal interests of the LCBG. In the classical chromaffin cell system we have found that the nicotinic receptor regulating secretion of chromaffin granule contents by exocytosis is itself controlled by sigma opiate receptors. Chromaffin granules contain dopamine monooxygenase (DBH) which is responsible for synthesis of noradrenaline. We found that external ATP regulates DBH function in chromaffin granules by a mechanism unrelated to effects of proton pumping. A major chromaffin granule membrane protein cytochrome b561 has been cloned and localized to human chromosome 17q11-qter. When the chromaffin cell is stimulated more granule contents are synthesized but cytochrome b561 levels maintained. The new vesicles apparently have greater secretory quanta. Islets of Langerhans secrete insulin in response to glucose and other stimuli. We found that both glucose- and muscarinic agonist-potentiated secretion is mediated by calcium from both intracellular and extracellular sources. Glucose sensing mechanisms in neonatal rat islets are non-functional, and are thought to model changes occurring during type II diabetes. However, glucose sensitivity can be prematurely induced by exposure of the islets to prolactin. Endothelial cells have been shown to form relatively impermeable sheets in culture, and to join together by lucifer yellow-delimited gap junctions. Albumin has a modulatory effect on endothelium permeability which is species specific. Ascorbic acid accumulation and recycling is enhanced in activated human neutrophils. In situ kinetics of ascorbate utilization can be used to evaluate true requirements for man. A toxic effect of ascorbate is the suppression of insulin secretion from isolated islets, and may be the basis for the tight control of blood levels in intact organisms. Calcium action during exocytotic control of blood levels in intact organisms. Calcium action during exocytotic membrane fusion may be controlled by synexin (annexin VII), and we have recently localized human synexin gene to chromosome 10q21.1-21.2. The structure of the gene varies from other members of the annexin gene family. In anticipation of studies with a synexin knockout mouse we have cloned synexin from mouse, and have characterized the mouse synexin genomic sequence. It is virtually identical to the human case. Calcium metabolism in muscle has also been studied with the local anesthetic tetracaine. Rynaodine receptors have also been characterized in lobster skeletal muscle. Finally, beta amyloid ion channels have been further characterized and evaluated as possible toxic agents in the genesis of Alzheimer's disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 22001 03

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vitamin C: Biochemistry, Molecular Biology and Human Requirements

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Mark A. Levine	Senior Investigator	LCBG:NIDDK
Other:	Kuldeep Dhariwal	Senior Staff Fellow	LCBG:NIDDK
	Jae Park	IRTA	LCBG:NIDDK
	Richard Welch	IRTA	LCBG:NIDDK
	Yaohui Wang	IRTA	LCBG:NIDDK
	Yukio Yamada	IRTA	LCBG:NIDDK

COOPERATING UNITS (if any)

L. Cantilena (USUHS, Bethesda), C. Cantilena (Transfusion Medicine, CC, NIH), L. Helman (PB, NCI, NIH) and M. Vasquez (PB, NCI, NIH), K. Kirk (LBC, NIDDK, NIH), A. Crossman (LBC, NIDDK, NIH), S. Chanock (PB, NCI, NIH)

LAB/BRANCH

Laboratory of Cell Biology and Genetics

SECTION

Section on Cell Biology and Biochemistry

INSTITUTE AND LOCATION

NIDDK:NIH, Building 8, Room 403, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	5	-

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The recommended dietary allowance for vitamin C (ascorbate) is based on preventing the deficiency disease scurvy. Optimal vitamin C requirements are unknown. We proposed that optimal requirements can be determined using the concept of in situ kinetics.

In situ kinetics has biochemical and clinical components. The goal of the biochemical component is to determine vitamin C molecular function in relation to its concentration. For these studies vitamin C function is investigated in human fibroblasts, lymphocytes, and neutrophils. To determine how intracellular concentration is regulated, vitamin transport has been characterized. Ascorbate transport is concentration dependent, saturable, mediated by a sodium dependent carrier, and inhibited by newly synthesized vitamin C analogs. Oxidized ascorbate, dehydroascorbic acid is transported separately by GLUT I and immediately reduced intracellularly to ascorbate. Two distinct proteins have been isolated which mediate intracellular reduction in neutrophils. Cloning of these proteins is underway. These studies indicate that vitamin C function in neutrophils may be to protect neurtophils from their own oxidants. One function of vitamin C in lymphocytes may be regulation of regulation of gene transcription. The overall findings show that kinetics for vitamin C function in situ can be determined in relationship to vitamin concentration in situ.

The clinical component of in situ kinetics is to determine how vitamin concentrations are achieved in normal humans as a function of dose and whether concentrations human achieve are in the range reguired for molecular functions of the vitamin. A clinical trial has been completed in normal men which investigated the relationship between vitamin C dose and its concentration in plasma and tissues, bioavailability, urinary excretion, and potential adverse effects. A clinical trial in women is underway.

Based on biochemical and clinical in situ kinetics, we can recommend for the first time an optimal vitamin C requirement in health humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 23,330-16 LBP

PERIOD COVERED
October 1, 1993, through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The permeability barriers of gram-negatives and mycobacteria to antibiotics and chemotherapeutics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William G. Coleman, Jr., Ph.D.	Research Microbiologist	LBP NIDDK
Others:	Lishi Chen, Ph.D.	Visiting Associate	LBP NIDDK
	Li Ding, Ph.D.	Visiting Fellow	LBP NIDDK
	Keith Crawford, Ph.D.	IRTA Fellow	LBP NIDDK
	Dorothy Paul	Special Volunteer	LBP NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.8	4.0	0.8

CHECK APPROPRIATE BOX(ES

(a) Human subjects (b) Human tissues [✓ (c) Neither]
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The *rfaD* gene and its gene product, ADP-L-glycero-D-mannoheptose 6-epimerase have been defined and reported by my laboratory. An efficient *rfaD* gene expression system and a two-step purification protocol facilitated the purification of the *rfaD* gene product to greater than 95% homogeneity. Physicochemical studies of the epimerase indicate that it is an NAD-containing glycosylated enzyme of 240 KD. Stereochemical, kinetic and structural studies of the epimerase are on-going. Procedures for crystallization of the epimerase have successfully yielded three crystal forms of the enzyme adequate for preliminary X-ray diffraction analysis. We have demonstrated structural and functional homologies of the *rfaD* gene product of *Brucella abortus* and the nonenteric pathogen *Pseudomonas aeruginosa* to its *E. coli* K-12 counterpart.

We demonstrated the function of the *rfaC* gene product as heptosyl transferase I, and we have provided evidence for its significant interspecific similarity and identity. We developed a preparative polyacrylamide gel electrophoretic purification protocol for the heptosyl transferase I. Physicochemical studies of the *rfaC* product are on-going.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 23,580-31 LBP

PERIOD COVERED

October 1, 1993, through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mammalian Transposons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Anthony V. Furano, M.D.	Medical Officer (Research) and Chief, Section on Genomic Structure and Function	LBP NIDDK
Others:	Karen Usdin, Ph.D.	Visiting Associate	LBP NIDDK
	Mary Zavanelli, Ph.D.	IRTA	LBP NIDDK
	Renée Howell, Ph.D.	IRTA	LBP NIDDK
	Eric Cabot, Ph.D.	IRTA	LBP NIDDK
	Kerry Woodford	Special Volunteer	LBP NIDDK

COOPERATING UNITS *(if any)*

Dr. Francois Catzeflis, Institute of Evolutionary Science, Montpellier, France;
Dr. Michael Seidman, Otsuka Pharmaceuticals, Rockville, MD

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Genomic Structure and Function

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.2	4.2	1.0

CHECK APPROPRIATE BOX(ES

(a) Human subjects (b) Human tissues √ (c) Neither
  (a1) Minors
  (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

L1 retrotransposable elements are high copy number genomic parasites found in mammals and are thought to cause as many as 0.2% of the genetic defects in humans. L1 elements are 6-7 kb long and contain two protein encoding genes. A regulatory region is at the left end of the element, and a guanine-rich polypurine:polypyrimidine sequence is near the right end. The protein encoding sequences of mammalian L1 families are highly conserved, but the regulatory sequences are completely distinct. We discovered the existence of ancient L1 families in mammalian genomes and our studies of them and their modern counterparts showed that extensive amplification of L1 elements has been episodic and recurrent during mammalian evolution, and that L1 amplification events provide very robust phylogenetic information. We previously showed that the regulatory region of the rat L1 element strongly stimulates the activity of a gene fused to it which was the first evidence that L1 DNA is not just some non-functional "junk" DNA. Although the DNA of the regulatory region can form specific complexes with nuclear DNA-binding proteins, the L1 regulatory region is not a typical transcriptional activator sequence since it stimulates gene activity by acting as an activator of protein translation. Dr. Usdin has extended her work on non-B (abnormal) DNA structures. Her group has shown that an unusual nucleic acid structure is a highly conserved feature of the 3' untranslated region of L1 elements which strongly indicates that is vital for the biological activity of L1 elements. She also has discovered a new class of very strong DNA synthesis arrest sites that is abundant in the genomes of many organisms. The molecular basis of the arrest is the formation of a K^+ dependent intrastrand tetrahelical structure. She speculates that the arrest of DNA synthesis or other possible effects of the tetrahelical structure could account for the genetic instability that is associated with these sequence motifs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 23,900-03 LBP

PERIOD COVERED
October 1, 1993, through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemistry and Function of Microtubules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jan Wolff, M.D., Ph.D.	Medical Officer and Chief, Section on Endocrine Biochemistry	LBP NIDDK
Others:	Dan Sackett, Ph.D.	Expert	LBP NIDDK
	Shobha Uppuluri, Ph.D.	Visiting Associate	LBP NIDDK
	Leslie Knipling	Biologist	LBP NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Endocrine Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES

◊ (a) Human subjects ◊ (b) Human tissues X (c) Neither
◊ (a1) Minors
◊ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A number of tubulin domains have been studied by a variety of methods.

(1) We have found that benzophenanthridines constitute a new class of antimicrotubule agents. Chelidonine interacts at the colchicine site whereas sanguinarine and chelerythrine interact with tubulin through reversible pseudobase formation by nucleophilic attack.

(2) Colchicine binds as the interface between α and β tubulin with primary binding on β and secondary binding on α. There are three α-domains involved in binding and indirect evidence suggests that it is the B ring of colchicine that is in contact with α tubulin.

(3) Colchicine binding leads to long range conformational effects on β-tubulin that lead to the unfolding of the C-terminus which, in turn, prevents addition of new dimers during polymerization and may explain the coupling of hydrolysis of the GTP cap to polymerization.

(4) Very low (<1M) concentration of urea modify numerous properties of tubulin in the order decreased polymerization > decreased GTPase > enhanced fluorescence of a colchicine analogue > decreased proteolytic accessibility > enhanced ANS fluorescence. These can be shown to be independent regions and suggest multistep unfolding of the protein. Physical correlates are now being sought.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 24,140-28 LBP

PERIOD COVERED

October 1, 1993, through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of the Tryptophan Synthase Multienzyme Complex

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Edith Wilson Miles, Ph.D.	Research Chemist and Chief, Section on Enzyme Structure and Function	LBP NIDDK
Others:	Syed A. Ahmed, Ph.D.	Senior Staff Fellow	LBP NIDDK
	Sergei Ruvinov, Ph.D.	Visiting Fellow	LBP NIDDK
	Utpal Banik, Ph.D.	Visiting Fellow	LBP NIDDK
	Li-hong Yang, Ph.D.	Visiting Fellow	LBP NIDDK

COOPERATING UNITS *(if any)* Drs. D.R. Davies, C.C. Hyde & K. Parris, LMB, NIDDK; C.C. Hyde, LSBR, NIAMS; P. McPhie, LBP, NIDDK; A. Ginsburg & D.P. Remeta, LB, LHLBI; P. Brzovic & M.F. Dunn, Univ. of California, Riverside; A. Mozzarelli & G.L. Rossi, Univ. of Parma, Italy; K.S. Anderson & Y.A. Kim, Yale Univ., New Haven, CT; I. Schlicting, Max Planck Institute, Heidelberg, Germany; D.L. Sackett, LBP, NIDDK; Drs. D.-M. Zhu & P.B. Chock (LB, NHLBI)

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Enzyme Structure and Function

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	

CHECK APPROPRIATE BOXES

◇ (a) Human subjects ◇ (b) Human tissues ✓ (c) Neither

◇ (a1) Minors

◇ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)* We are using the tryptophan synthase multienzyme $\alpha_2\beta_2$ complex as a model system for investigating how enzymes interact, communicate, and channel reaction intermediates. Our previous X-ray crystallographic studies of the wild type tryptophan synthase $\alpha_2\beta_2$ complex from *Salmonella typhimurium* reveal that the active sites of the α and β subunits are 25 Å apart and are connected by a tunnel. We are using the crystal structure as the basis for engineering mutations designed to alter the tunnel and to alter subunit interaction and communication. During the past year we have used several approaches to analyze wild type and mutant enzymes. (1) Alterations in the tunnel at positions 170 and 280 of the β subunit have been investigated by analyses with a fluorescent probe (Nile Red), by rapid kinetic studies, and by X-ray crystallography. Replacing β Cys-170 by a bulky tryptophan results in partial or total blocking of the tunnel. Our results indicate that β site ligands and α site ligands convert the tunnel from an open form to a closed form. (2) Studies of the effects of alcohol cosolvents on the spectroscopic and catalytic properties of the β subunit in the $\alpha_2\beta_2$ complex show that cosolvents stabilize the open form of the β subunit in the $\alpha_2\beta_2$ complex. (3) X-ray crystallographic studies have refined the structures of the wild type $\alpha_2\beta_2$ complex and of Schiff base intermediates of a mutant $\alpha_2\beta_2$ complex (K87T) to ~2 Å resolution. The results reveal ligand-induced conformational changes. (4) The allosteric activation of the α subunit in the $\alpha_2\beta_2$ complex by a β site ligand, L-serine, has been analyzed by steady-state and rapid kinetic studies using an $\alpha_2\beta_2$ complex with a mutation in the β subunit (K87T). The results provide evidence that the Schiff base of aminoacrylate is the key intermediate at the β site that triggers activation of the α subunit in the $\alpha_2\beta_2$ complex. (5) Thermally-induced unfolding of the separate α and β subunits and $\alpha_2\beta_2$ complex has been analyzed by several techniques: UV-visible and circular dichroism spectroscopy, activity measurements and differential scanning calorimetry. The stepwise unfolding of the β subunit is modulated by the presence of the α subunit. These results permit correlation of thermodynamic and structural information.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 24,150-23 LBP

PERIOD COVERED

October 1, 1993, through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Noncovalent Intermolecular Interactions in Biochemistry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Allen P. Minton, Ph.D.	Research Chemist and Chief, Section on Physical Biochemistry	LBP NIDDK
Others:	Saleh Darawshe, Ph.D.	Visiting Fellow	LBP NIDDK
	Jianqing Wu, Ph.D.	IRTA	LBP NIDDK

COOPERATING UNITS *(if any)* J. C. Parker, Univ. of North Carolina, Chapel Hill; P. B. Dunham, Syracuse Univ.; G. B. Howlett, Univ. of Melbourne, Australia

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Physical Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.8	2.8	

CHECK APPROPRIATE BOXES

◇ (a) Human subjects ◇ (b) Human tissues ✓ (c) Neither

◇ (a1) Minors

◇ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanism by means of which volume-sensitive membrane ion transporters respond to alterations in intracellular content has been studied experimentally and theoretically.

A computer program for rapid simulation of many types of experiments that may be performed on the analytical ultracentrifuge has been developed.

A new technique for quantification of concentration gradients of multiple proteins in a mixture at sedimentation equilibrium has been developed.

Computer programs employing novel algorithms for the global analysis of multiple absorbance gradients of two component solutions at sedimentation equilibrium in the context of models for self- and heteroassociation have been developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 24,590-23 LBP

PERIOD COVERED
October 1, 1993, through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Interactions of Biologically Important Macromolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Harry A. Saroff, Ph.D.	Research Chemist (Intermittent)	LBP NIDDK
Other:	Elemer Mihalyi, M.D., Ph.D.	Special Volunteer	LBP NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Physical Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES

(a) Human subjects (b) Human tissues [✓ (c) Neither]
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cooperative binding systems are being studied taking into account site or subunit interactions, ligand interactions, aggregation and redistribution in proteins, and model systems. Methods are being developed to evaluate reasonable values for the parameters describing these systems, particularly those involving the action of fibrinogen and the processes of protein-DNA interactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 24,709-13 LBP

PERIOD COVERED
October 1, 1993, through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Polyamine Biosynthesis and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Celia White Tabor, M.D.	Medical Officer (Research)	LBP NIDDK
Others:	Herbert Tabor, M.D.	Supervisory Medical Officer (Research) Chief, Section on Pharmacology, LBP; and Chief, Laboratory of Biochemical Pharmacology	LBP NIDDK
	David Balasundaram, Ph.D.	Visiting Associate	LBP NIDDK
	Nobuko Hamasaki, Ph.D.	IRTA	LBP NIDDK

COOPERATING UNITS *(if any)*
Drs. R. B. Wickner and J. Dinman, GSE, LBP, NIDDK

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	

CHECK APPROPRIATE BOX(ES

(a) Human subjects (b) Human tissues √(c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The polyamines putrescine, spermidine, and spermine are major cellular components af all living cells and have been shown to be involved in many systems related to growth and differentiation. Our current and older studies have been directed at learning how these polyamines are synthesized and regulated and their physiological function *in vivo*. As part of these studies we are currently concentrating on the biochemistry, regulation, and genetics of these amines in *Saccharomyces cerevisiae*. In our past studies, we prepared mutants in the biosynthetic pathways and showed that cells that were deprived of spermidine or spermine were unable to grow, to sporulate, or to maintain the virus-like RNA killer plasmids of yeast. Our current studies have demonstrated three additional *in vivo* functions: (1) Spermidine protects yeast from oxidative damage due to superoxide formation. (2) Spermidine is necessary for the survival of mitochondria, which are lost in amine-deficient cells even in air. (3) We have also shown an unusual and unexpected effect of spermidine on protein synthesis *in vivo*. With the use of a model system for the study of ribosomal frameshifting, described by Wickner and Dinman, and in collaboration with them, we have shown that polyamines are essential for the maintenance of the correct translational efficiency of +1 ribosomal frameshifting. Ribosomal frameshifting is of particular importance in the development of retroviruses and -1 ribosomal frameshifting is known to be important in the "gag-pol" type of protein synthesis in the L-A dsRNA system of yeast studied by Dinman and Wickner and in the Ty transposon system. In very recent studies we have shown that the amount of ribosomal frameshifting is controlled by the putrescine/spermidine ratio, i.e. not by spermidine alone. Other studies have been concerned with further studies on the enzymes involved in the biosynthetic pathway and their regulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 24,940-21 LBP

PERIOD COVERED
October 1, 1993, through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Yeast RNA Virology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Reed B. Wickner, M.D.	Medical Officer, USPHS and Chief, Section on Genetics of Simple Eukaryotes	LBP NIDDK
Others:	Tsutomu Fujimura, Ph.D.	Visiting Scientist	LBP NIDDK
	Jonathan D. Dinman, Ph.D.	Staff Fellow	LBP NIDDK
	Rosaura P. C. Valle, Ph.D.	Visiting Fellow	LBP NIDDK
	Juan Carlos Ribas, Ph.D.	Visiting Fellow	LBP NIDDK
	Kathleen Carroll, Ph.D.	IRTA Fellow	LBP NIDDK
	Daniel Masison, Ph.D.	IRTA Fellow	LBP NIDDK
	Yasuyuki Otake, Ph.D.	Guest Researcher	LBP NIDDK

COOPERATING UNITS *(if any)* N. Sonenberg, McGill Univ.; A. Steven, LSB, NIAMS; T. Baker, Purdue Univ.; J. Kwon-Chung, NIAID

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Genetics of Simple Eukaryotes

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.6	7.6	

CHECK APPROPRIATE BOX(ES

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The L-A double-stranded RNA virus of Saccharomyces cerevisiae encodes its major coat protein (Gag) and a multifunctional Pol domain expressed only as a Gag-Pol fusion protein formed by a -1 ribosomal frameshift. The frameshift event occurs by a mechanism identical to that used by retroviruses to form their own Gag-Pol fusion proteins. We have found that the 5S rRNA affects the efficiency of frameshifting, as do several other cellular genes.

The L-A virus decaps cellular mRNAs in order to form decoys to distract the cellular *XRN1*- encoded 5' exoribonuclease specific for uncapped RNAs from degrading the viral mRNA. The yeast *SKI2,3,8* system represses viral replication by limiting the tranlation of non-poly(A) mRNAs, such as that of L-A. We propose that the *SKI2,3,8* system prepares 60S ribosomal subunits to make them require interaction with the 3' poly(A) before they will bind to the 40S subunit waiting at the initiator AUG.

We find that of 27 genes (MAK genes) required for propagation of M1 dsRNA (a satellite of L-A), mutants in 20 are deficient in 60S ribosomal subunits. Some encode proteins of the large ribosomal subunit (*MAK7 = RPL4A; MAK8 = RPL3; MAK18 = RPL41B*) while others do not. We suggest that non-poly(A) mRNAs are at a disadvantage in competing for 60S subunits.

We describe the first dsRNA virus of a human pathogenic fungus, *Blastomyces dermatitidis*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 24,941-03 LBP

PERIOD COVERED
October 1, 1993, through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Membranes Cytoskeleton and Secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jan Wolff, M.D., Ph.D.	Medical Officer and Chief, Section on Endocrine Biochemistry	LBP NIDDK
Others:	Leslie Knipling	Biologist	LBP NIDDK

COOPERATING UNITS *(if any)* Dr. M. Bifulco, Univ. of Naples; Dr. E. Miles, LBP, NIDDK; Dr. Y. Pommier, DCT, NCI; Dr. N. Nguyen, DCB, CBER, FDA

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Endocrine Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES

(a) Human subjects (b) Human tissues x (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The properties and functions of a protein whose activity appears to depend on prenylation is being studied in thyroid cells and brain tissue for its role in microtubule anchoring to the plasma membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK24,942-02 LBP

PERIOD COVERED
October 1, 1993, through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Thermodynamic and Kinetic Studies of Protein Structure and Enzymic Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter McPhie, Ph.D.	Research Chemist	LBP NIDDK

COOPERATING UNITS *(if any)*
Dr.Edith W. Miles, LBP; Dr. William Jakoby, LBM; Dr. S-y Cheng, LMB, NCI; Dr. Paul Plotz, ARB, NIAMS; Dr. D. Mahadevan, LCMB, NCI

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Enzyme Structure and Function

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES

(a) Human subjects (b) Human tissues ✓ (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This laboratory is engaged in studies on protein structure and the mechanism of protein folding. The main subject of research is swine pepsinogen, a monomeric protein of molecular weight= 39,630, which is stable at pH's between 6 and 8.5. Below pH 6 pepsinogen activates itself by proteolytic loss of its first 44 amino acids, to produce an enzymatically active protein, pepsin. Pepsin is stable only at pH's below 6. Pepsin and pepsinogen are unfolded by exposure to high pH, temperature or concentrations of denaturants, such as urea. However, unfolded pepsinogen can refold to its normal structure, when returned to native conditions, whereas pepsin cannot. I am interested in the mechanism of this refolding reaction and how the difference in sequence influences the refolding of the two proteins. We have used techniques such as ultra-violet, circular dichroic and fluorescence spectroscopies, together with chemical modification and peptide chemistry, to characterize the structures of the native and unfolded species. We have used rapid kinetic techniques, such as stopped-flow and T-jump, to detect partly folded forms in the folding reaction; their structures have been partially determined and the nature of the chemical reactions which separate them from the native and unfolded forms investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 24,943-01 LBP

PERIOD COVERED

October 1, 1993, through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prions of Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Reed B. Wickner, M.D.	Medical Officer, USPHS and Chief, Section on Genetics of Simple Eukaryotes	LBP NIDDK
Others:	Daniel C. Masison, Ph.D.	IRTA	LBP NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Genetics of Simple Eukaryotes

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES

◇ (a) Human subjects ◇ (b) Human tissues ✓ (c) Neither

◇ (a1) Minors

◇ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A prion is an infectious protein, a concept developed in studies of scrapie of sheep and of kuru and Creutzfeldt-Jakob disease of humans. An altered form of a protein, on entering a new individual, can convert the normal form into this altered form. This results in loss of the normal form and accumulation of the altered (prion) form, either of which can have physiological effects.

I have confirmed that [URE3] requires *URE2* for its propagation by a new method using the *URE2* gene on a shuttle vector. I have also shown that [URE3] can be cured by growth of cells on rich medium containing 5 mM guanidine HCl, but that the cured cells can again become [URE3]. Finally, overproduction of Ure2p results in a 100-fold increase in the frequency with which cells become [URE3]. These are the properties expected of a yeast prion, not those expected if [URE3] is a DNA or RNA replicon.

The [PSI] non-Mendelian element of yeast increases the efficiency of suppression of nonsense and frameshift mutations by classical suppressor tRNAs. [PSI] has similar properties to [URE3], making it likely that [PSI] is also a prion. [PSI] requires the chromosomal gene *SUP35* for its propagation. Recessive mutants of SUP35 have the same phenotype as does the presence of [PSI]. [PSI] can be reversibly cured by either 5 mM guanidine HCl or by high osmotic strength. Finally, overproduction of Sup35p results in a 100-fold increase of the conversion of cells from [psi-] to [PSI+].

My proposal that [URE3] and [PSI] are prions of yeast has both provided the first model systems for mammalian prion diseases and supported the notion of prion as protein - only infectious elements.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25011-19 LCB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Protein Folding

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Hiroshi Taniuchi	Chief, Section on Protein Chemistry and Conformation	LCB, NIDDK
Others:	Alice Hawley	Chemist	LCB, NIDDK
	Ying Shi	Visiting Fellow	LCB, NIDDK
	Greg Charles	Biological Aid	LCB, NIDDK
	Mona Sha	Summer IRTA	LCB, NIDDK

COOPERATING UNITS (if any)

Structural Biophysics Section, Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute.

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Protein Chemistry and Conformation

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL STAFF YEARS 3.41	PROFESSIONAL: 2.5	OTHER: 0.91

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the previous years we have assigned four core domains of cytochrome c and their folding order. The previous studies have also suggested that the folding of cytochrome c may be driven by the core domain-domain interaction. Since 1988 several groups of other investigators have identified folding intermediates of different proteins which appear to be consistent with our concept of the core domains. Thus, the core domain-domain interaction may be general in driving the folding of globular proteins. To know whether or not the core domain-domain interaction differs from the the known types of non-covalent interactions we have determined the differences in their thermodynamic and kinetic effects of substitution with norvaline (Nva) between Leu32 and Leu35 of the horse cytochrome c three-fragment complex in the previous years. The complex consists of a heme fragment of residues 1 to 5, (1-25)H, and two apofragments (28-38) and (39-104). It resembles native cytochrome c except for residues 39 to 55 which are flexible. We have now analyzed and interpreted the data using complex models. The results suggest for the first time that the difference in the standard Gibbs energy change, ddGs, associated with the equilibrium constant of fragment (28-38) with the ferric or ferrous complex qualitatively correlates with the difference in the activation Gibbs energy, dGa, associated with the direct dissociation of fragment (39-104). These ddGs and dGa also qualitatively correlate with the difference in the heat stability of the 695 nm band of the ferric complex, a band known to be indicative of the Fe-S bond. These correlations have been interpreted as suggesting that some propagative extra non-covalent interactions may be generated by the packed atomic groups in the hydrophobic core to stabilize the ground state. These interactions may have characteristics such that the stabilizing energy generated by them would be perturbed much more by the Leu32 Nva substitution than the Leu35 Nva. The perturbation of these interactions imposed by the removal of the gamma-methyl group of Leu32 would propagate itself through the core and affect the stability of the Fe-S bond and the binding strength of (39-104). These properties are consistent with the core domain-domain interaction. Furthermore, the analysis, combined with other evidence, appears to suggest that these propagative non-covalent interactions in the core are likely to differ from van der Waals interactions, hydrophobic energy, hydrogen bonds and charge-charge interactions. To map the core side chains involved in these propagative non-covalent interactions studies of yeast iso-2-cytochrome c using site directed mutagenesis is in progress. To date eight mutants have been or are being generated. To precisely identify the core domains of cytochrome c NMR studies are also in progress using two-dimensional NOESY, COSY, and TOCSY spectra in collaboration with the structural Biology Section, LBC, NHLBI.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25016-20 LCB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Trans-Acting Factors Controlling Globin Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jin Li	IRTA Fellow	LCB, NIDDK
Others:	Constance T. Noguchi	Chief, Molecular Cell Biology Section	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL MAN-YEARS: 1.2	PROFESSIONAL: 1.2	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The developmental and tissue-specific control of hemoglobin synthesis is a complex process involving two switches which control the expression of specific hemoglobin genes; for example, the beta-like globin genes switch from embryonic (epsilon) to fetal (gamma) to adult (beta). A number of trans-acting proteins and cis-acting DNA elements have been found to be part of the switching machinery, including the silencer located in the region between -250 and -300 bp 5' to the epsilon-globin gene promoter, which was first discovered in this Laboratory. Further characterization of the silencer will provide not only answers to basic questions regarding the regulation of hemoglobin gene expression, but also better understanding of the problems related to hemoglobinopathies. In order to further characterize the DNA sequences functioning co-operatively with the silencer, we have constructed a plasmid, pE6000, which contains the epsilon-globin gene promoter, its 5' upstream flanking sequences up to 6021 bpo and a reporter gene. This construct has been deleted progressively from the far 5' end to give a series of nested deletion mutants with various lengths of the upstream sequence. Some of these constructs have been transiently transfected into K562, a human erythroleukemia cell line and different levels of expression of the reporter gene, luciferase gene, were observed with different 5' upstream deletions. In transient transfection assays, deletion of 5' sequences between -935 and -5824 bp and between -2832 and -5824 bp changes the epsilon-globin gene promoter activity. This result indicates that DNA sequences located in the region between -900 and -6000 bp may have multiple control elements to regulate epsilon-globin gene expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25021-19 LCB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Sickle Cell Anemia: The Intracellular Polymerization of HbS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Constance Tom Noguchi	Chief, Molecular Cell Biology Section	LCB, NIDDK
Others:	Griffin P. Rodgers	Chief, Molecular Hematology Section	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS (if any)
BEIB, NIH (R. Chadwick); Howard University (W. Poillon); University of Pennsylvania (C. Dong); George Washington University (L. Lessen); Paris Consortium (M. Redelsburger, R. Girot, J. Elion); Nippon Medical School, Tokyo, Japan

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Cell Biology

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL STAFF YEARS 0.3	PROFESSIONAL: 0.3	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hemoglobin S polymerization is the primary determinant of the abnormal rheology associated with sickle cell anemia, which is characterized by chronic hemolysis and acute painful crises. Measurements of abnormal cell rheology in sickle cell anemia continue to provide correlations with clinical manifestations although the detailed mechanisms leading to such clinical endpoints are not well understood. The profound effect of hemoglobin S polymerization on cell rheology has given rise to a variety of therapeutic strategies designed to reduce the extent of intracellular polymerization. We have examined the solubility of hemoglobin S mixtures with hemoglobins A, A2 and F at varying oxygen saturations to determine the extent of hemoglobin S polymerization under physiologic conditions. A detailed analysis provides the means to predict the maximum extent of polymerization within the sickle hemoglobin containing erythrocyte. Equilibrium solubility of sickle hemoglobin mixtures confirm previous findings that the sparing effect on hemoglobin S polymerization of hemoglobin F is greater than the sparing effect of hemoglobin A in mixtures of hemoglobin S. The current study also demonstrates explicitly the polymerization behavior of hemoglobin S and hemoglobin F mixtures at increasing ligand concentrations.

The theoretical model based on experimental data of polymerization of hemoglobin mixtures was used to analyze data obtained from the Parisian Prospective Study on Sickle Cell Disease in children with sickle cell anemia during the first two years of life. These data indicated that 3 of 21 children had a significantly greater predicted polymerization tendency due to early decreases in hemoglobin F. These individuals will be studied prospectively to ascertain the relationship among polymerization tendency and various clinical manifestations of sickle cell disease. Observations obtained from 2674 individuals with sickle cell anemia from the Cooperative Study of Sickle Cell Disease database indicated that changes in red cell indices result in continuous rise in polymerization tendency during the first twelve years of life.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25025-17LCB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Mechanism of Antigen-Antibody Interaction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Hiroshi Taniuchi	Chief, Section on Protein Chemistry and Conformation	LCB, NIDDK

Others:

COOPERATING UNITS (if any)
OSD, NICHD, Dr. Paola Rizzo

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Protein Chemistry and Conformation

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL STAFF YEARS .5	PROFESSIONAL: .5	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It has been known with polyclonal antibodies that the antigen-binding suppresses the hydrogen-exchange of the antibodies even if the antigen is small. In the previous years we have determined the number of the amide protons whose exchange is suppressed by the antigen using three monoclonal antibodies (mAb) to yeast iso-1 cytochrome c and the Fab fragment of one of them. The results have suggested a possibility that the antigen-binding may increase the stability of the hydrophobic cores of the Fab domains which are remote from the antigen combining site. If this hypothesis is correct, the non-covalent interactions in the cores of the Fab domains fragment, particularly those of the variable domains of the heavy and light chains (VH and VL, respectively) and also possibly those of the VH-VL interface, may influence the antigen binding (based on the action-reaction principle). By inspection of the available x-ray structures of the antigen-antibody complexes we have realized that the antigen always contracts the particular geometrical site of the antigen combining surface which is related to the VH-VL interface. We call this site as the antigen-combining surface center. We have speculated that the interaction of the antigen with the residues located at the antigen combining surface center might be related to the mechanism in which the antigen binding stabilizes the cores of the Fab domains and therefore important to generate the affinity of the antigen to the antibody. To test this idea we have used mAb 2-96-12 to yeast iso-1-cytochrome c, one of the above mAbs. In the previous years we have deduced the amino acid sequences of the VH and VL from the cDNAs, constructed molecular models of the VH-VL complex and identified Arg at position 95 of the VH as one of the residues located at the antigen - combining surface center. A baculovirus transfer vector in which the cDNAs encoding the light chain and the VH and the first constant domain of the heavy chain of mAb 2-96-12 were inserted was constructed. Using this vector and the virus DNA the recombinant viruses were generated to express the Fab (wild type) in the insect cells. The Arg 95 → Lys mutation of the heavy chain was accomplished using oligonucleotide - directed mutagenesis. The expressed wild type and mutant Fabs were purified from the culture media of the virus infected cells and characterized with respect to the immunological activity. Preliminary results appear to show that (a) the bioengineered Fab closely resembles the native mAb with respect to the affinity to the antigen and the fine specificity; (b) the heavy chain Arg 95 → Lys mutant Fab also exhibits the fine specificity similar to the native mAb; and (c) however, its affinity to the antigen is markedly decreased as compared with the wild type despite the fact that there should be no difference in the charge. Thus, the concept of the antigen combining surface center appears to be useful to identify the residues of mAb which serve as the starting point for the substitution studies of the antigen-antibody interaction before the x-ray structure is determined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 25028-15 LCB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Development of Non-Invasive Methods to Assess Sickle Cell Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Griffin P. Rodgers	Chief, Molecular Hematology Section	LCB, NIDDK
Others:	Hiroyuki Hiruma	Visiting Fellow	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS (if any)

Clinical Hematology Branch, NHLBI (A.W. Neinhuis); Transfusion Medicine, CC (H. Klein); BEIB (Eli Walker); Biometry Branch, NEI (M. Podgor); MRC Laboratory, Kingston, Jamaica (G. Serjeant); Nippon Medical School (Dr. N. Uyesaka)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been suspended.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25058-08 LCB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of Human Adult Beta-like Hemoglobin Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Delia Ching Tang	IRTA Fellow	LCB, NIDDK
Others:	Griffin P. Rodgers	Chief, Molecular Hematology Section	LCB, NIDDK
	David Ebb	Special Volunteer	LCB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Hematology

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL MAN-YEARS: 0.7 PROFESSIONAL: 0.7 OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have been investigating the molecular mechanisms which govern the tissue and developmental stage-specific expression of the adult beta-like globin genes. Our efforts have focused on establishing the combination of trans-acting factors and DNA cis-elements that determines the low level of expression of the adult beta-like globin genes in immature erythroid tissues. It is our hope that elucidating the mechanisms of globin gene regulation will facilitate the manipulation of hemoglobin synthesis in individuals affected by underexpressed or structurally abnormal globin genes.
Our current studies of hemoglobin ontogeny have employed the K562 human erythroleukemia cell line as a tissue culture model of globin gene expression. Previous studies of this cell line revealed an embryonic-fetal phenotype, which is unique for low level expression of the beta globin gene in the absence of beta globin transcription. This phenotype contrasts markedly with normal erythrocytes from all stages of development, where expression of beta globin far exceeds delta, ranging from a ratio of 75:1 in fetal tissues to 40:1 in normal adults.

Using transient transfection assays, we have been exploring the possibilities that both the reversal of normal relative levels of synthesis of delta and beta globin, and the overall low level of expression of the two adult genes in K562 cells are due to the differential binding of trans-acting proteins to upstream regulatory regions. DNA fragments from the 5' flanking regions of the delta and beta globin genes were cloned into expression vectors containing the CAT reporter gene (pSV$_0$CAT), then transiently transfected into the K562 cells. We have clearly demonstrated the existence of a negative transcription element in the -500 to -250bp region of the beta globin gene, consistent with previous studies by Berg (N.A.R., 1989) which identified two negative regulatory elements (BP-1 and BP-2) 5' to the beta globin gene. We also identified an important positive transcription element in the comparable region flanking the delta globin gene. Structural analysis suggests that a potential regulatory element with extensive homology to the beta globin BP-2 motif exists in the delta globin 5' flanking region. We suggest that the negative regulatory elements upstream from the beta globin gene play an important role in expression of the adult beta-like globin genes in immature erythroid tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25061-19 LCB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Human Erythropoietin Receptor in Transgenic Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Zi-Yao Liu	Visiting Associate	LCB, NIDDK
Others:	Constance T. Noguchi	Chief, Molecular Cell Biology Section	LCB, NIDDK

COOPERATING UNITS (if any)

Brown University School of Medicine, Providence, R.I. (A. Anagnostou); Cornell University School of Medicine, New York, NY (Dr. B. Weksler)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL MAN-YEARS: 1.2	PROFESSIONAL: 1.2	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Erythropoietin (Epo) is a primary regulator of erythropoiesis and functions by binding to its receptor (EpoR) on the surface of hematopoietic progenitor cells, followed by receptor-mediated endocytOsis of the Epo molecule. Recent research showed that Epo acts in vitro and EpoR is present on other cells such as neural cells - PC12 and SN6 cell lines; megakaryocytes; B lymphocytes and endothelial cells. Therefore, stimulation of erythropoiesis is not the exclusive physiological function of Epo. Human Epo receptor was cloned and sequenced in our laboratory. A 15 Kb clones genomic fragment was introduced into the mouse genome and 12 transgenic mouse lines were generated. Several hEpoR transgenic lines and normal mice were used for examining the tissue and developmental specificity of gene expression. Hematological parameters and tissue expression were measured in both normal and transgenic mice from different lines. Our results show that hematological parameters were within normal limits except for a suggestion of increased reticulocyte levels. Human EpoR transcripts were detected in hematopoietic tissues (bone marrow and spleen) and in the brain of transgenic mice. We further examined developmental specificity of EpoR gene in mice. In normal mice mEpoR transcripts in fetal liver were down-regulated through a peak value at day 12-13 to zero before birth. We found that endogenous EpoR transcripts were present in fetal brain and down-regulated during development. hEpoR transcripts in brain of transgenic mice demonstrated a persistent level throughout embryonic and adult stages. Based on the above discovery, to understand the biological significance of EpoR expression in brains of transgenic mice, we extended our study to the expression of EpoR in the different cell types, such as Hela (as negative control), K562, OCIM1, Human Umbilical Vein Endothelial cells, Immortalized HUVEC and Bone Marrow Endothelial cells. Human EpoR transcripts were found in HUVEC and BMEC but not the others. The transcripts of the above cells were quantitated by competitive PCR. We are now studying the localization of the EpoR within neural tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 25063-08 LCB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Effect of Hydroxyurea on Fetal Hemoglobin Synthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
PI: Griffin P. Rodgers Chief, Molecular Hematology Section LCB, NIDDK

Others:

COOPERATING UNITS (if any)
Department of Biochemistry, Rockefeller University (J. Manning); Division of Hematology, Cornell University Medical College (B. Weksler); Department of Pharmacology, INSERM, Paris (A. Boulet).

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL STAFF YEARS 0.2 PROFESSIONAL: 0.2 OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recent clinical trials have substantiated hydroxyurea (HU) to be a potent effector of hemoglobin F (HbF) production in patients with sickle cell disease. This augmentation in HbF levels in patients is associated with a decrease in relative beta synthesis and thus an inhibitory effect of HbF. In order to gain insight into the possible genetic mechanisms underlying these effects, we used cation exchange (CE) and reverse-phase high performance liquid chromatography (HPLC) to examine changes in the levels of each hemoglobin species. Samples from ten patients treated with HU were analyzed approximately twice each week. Within the first 50 days of HU treatment, HbF increased on average from 3.3% to 7%. The patients had an average initial ratio G-gamma/A-gamma of 0.72 which did not change with treatment. At baseline, approximately 15-20% of the fetal hemoglobin exists in the acetylated form (F_1) as determined by CE-HPLC, independent of subtype. With HU treatment, the total amount of F_0 and F_1 increases roughly proportional to the increase in F-reticulocyte numbers. Although the total HbA_2 remained constant during treatment, there was in some patients an increase in a fraction which migrated with HbA_2. In 2 patients treated with combination HU and erythropoietin, we found a further increase in both F_1 and this modified HbS fraction to levels approaching 5% and 10%, respectively.

Subsequently, analysis of this modified beta-globin polypeptide showed that the NH_2 was not blocked (Edman sequencing) and that the modifier had a mass of 300 close to the one of glutathione (307) (mass spectroscopic analysis). A dual strategy using deblocking and synthetic methods has been used followed by IEF analysis and confirmed the glutathione nature of the abduct. Chemically modified HbS may interfere with HbS polymerization and thus have an added clinical benefit acetylation of other proteins (e.g. histones) may be involved in the regulation of gene expression; naturally occurring gluathione adducts have only been reported very recently. We have subsequently used this unique property of sickle hemoglobin - glutathione adducts at Cyc-93(B) - to examine the function properties of a recombinant sickle hemoglobin. It is possible that their formation is related to metabolic effects of HU treatment or, as with acetylation, this modification may be indicative of induced fetal erythropoiesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25064-08 LCB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Cytogenetic Investigations of Patients with Genetically Determined Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Beverly White	Director, Cytogenetic Unit	LCB, NIDDK
Others:	Damrong Wangsa	Photographer, Scientific Technical	OD, CC
	Flanagan Whitsitt	Medical Technician	OD, CC

COOPERATING UNITS (if any)
Medical Genetics Program, CC (D. Parry); DE NICHD (L. Nelson); LN, NIA (M. Shapiro, D. Murphy, S. Rapoport).

LAB/BRANCH Laboratory of Chemical Biology

SECTION Molecular Forces and Assembly (Cytogenetics Unit)

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 1.5	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Unit conducted collaborative research projects, provided cytogenetic services for NIH patients, and participated in the NIH Medical Genetics Training and College of America Pathologists proficiency testing programs. Case studies were from protocols for short stature, premature ovarian failure (POF), Turner syndrome (TS), mental handicaps, recognized dysmorphic syndromes, neurological diseases, and neoplasia. Detection of aberrations which might lead to mapping of disease genes was a frequent indication of cytogenetic evaluation. Both traditional methods and fluorescent in-situ hybridization (FISH) were used to identify abnormalities.

Numerical and structural sex chromosomal abnormalities (SCA) were the most frequent variations among 186 new cases. Of 128 TS patients studied, 65% were nonmosaic 45,X and 35% were genetic variants. Studies of cognition, visuospatial ability, and CNS structural variation in 9 subjects were reported. Significant differences were observed only in nonmosaics. Additional brain metabolic and neuropsychological studies of TS are being reported. Separate comparison of nonmosaics and genetic variants with controls resulted in more accurate data for genetic counseling in TS.

Screening of 166 POF patients revealed SCA in 2.8%, and 3% were familial. From our previous report of familial POF, our molecular analysis of an X;autosome translocation, and review of other genetic studies of POF, we suspect there are 2 critical regions for ovarian failure; Xq13.3-q21.1 and xq26.1-q27. Molecular studies of these regions in familial POF may lead to development of DNA probes for presymptomatic diagnosis in these families or permit detection of microdeletions in cytogenetically normal patients with sporadic POF.

We are continuing to evaluate patients with childhood schizophrenia and Fragile X syndrome, and several of these studies are being published. Also, the NCHGR is now actively involved in cytogenetic research at NIH, and we plan to interact with their investigators in our studies and genetics training program activities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25074-05 LCB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Pcr Method To Analysis Alpha-Thalassemia Genotypes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Delia Ching Tang	IRTA Fellow	LCB, NIDDK
Others:	Griffin P. Rodgers	Chief, Molecular Hematology Section	LCB, NIDDK

COOPERATING UNITS (if any)
Mahidal University, Bangkok Thailand (Dr. Suthat Fucharoen)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Hematology

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL MAN-YEARS: 1.0 PROFESSIONAL: 1.0 OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The α-thalassemias are common genetic disorders that arise from reduced synthesis of the α globin chains of fetal ($\alpha_2\gamma_2$, HbF) and adult ($\alpha_2\beta_2$, HbA) hemoglobins. The normal human α globin gene includes the duplicated α genes (α_2 and α_1). All diploid cells have four α-chain genes (i.e., $\alpha\alpha/\alpha\alpha$). In α-thalassemia syndromes, α-globin synthesis is either diminished or absent due to either deletional or non-deletional abnormalities involving the α-globin genes. At present, for a variety of technical, logistic and economic reasons, large-scale carrier screening and appropriate ante-natal detection programs have not been established for the populations in which this syndrome occurs. Here, in our pilot studies, we have successfully established a simple, rapid, sensitive PCR assay for detecting α gene deletion using DNA from fresh blood cells from α-thalassemia patients. We have demonstrated that deletion of hemoglobin gene α_1 or α_2 could be determined using three sets of primers (two sets for $\alpha 1$ and $\alpha 2$ gene respectively and one set for β-actin as internal control) from α-thalassemia syndrome patients by differential PCR. Using this method we have tested hemoglobin α gene status from 9 normal subjects and 7 α-thalassemia patients. Results clearly demonstrated that different extent of α genes deletion in these patients could be differentiated by measuring the band intensity of target genes ($\alpha 1$, $\alpha 2$) and reference gene (β-actin) after PCR amplification and gel electrophoresis. We have also been studying on the PCR mediated color complementary assay, which could serve as a quicker, sensitive method to detect gene deletion, omitting gel electrophoresis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25076-05 LCB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Characterization of the ε-Globin Silencer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Betty Peters	Special Volunteer	LCB, NIDDK
Others:	Constance Noguchi	Chief, Molecular Cell Biology Section	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Molecular Cell Biology Section

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hemoglobin switching is an example of tissue specific and temporal regulation of gene expression. In humans, embryonic globin chains are expressed during the first trimester of gestation followed by a switch to the expression of fetal globin chains early in the second trimester and adult globin chains after birth. Previous worked performed in the Laboratory of Chemical Biology has identified a silencer 200 to 400 bp upstream of the epsilon (e) globin gene which has been implicated in suppressing epsilon globin gene expression during the fetal and adult stages. Our work has focused on characterizing proteins binding to the silencer and the functional activity of the binding of trans-acting factors to the silencer. We are in the process of elucidating the mechanism(s) that trans-acting factors interacting with the silencer utilize in order to regulate e-globin transcription. We are also studying the interactions of the silencer with other regulatory elements controlling e-globin gene expression. Using DNase I footprinting, we have identified several sites of protein binding to the silencer. The major protected region shares a high percentage of homology with the binding sites of an erythroid specific transcription factor GATA-1 and a ubiquitous silencer binding protein, YY1. Using gel mobility shift assays with K562 nuclear extract, YY1 protein and a probe bracketing the major protected region and competitor DNA with mutations in the sites homologous to GATA-1 and YY1 binding sites we have demonstrated that a protein, likely GATA-1 binds to the GATA site in the silencer and that another protein, likely YY1, binds to the silencer. We are currently in the process of examining the activity of GATA-1 and YY1 on the expression of beta-like globin genes and determining the mechanism by which GATA-1 and YY1 binding to the silencer inhibits epsilon globin gene expression. Using transient expression assays we have demonstrated that the epsilon globin silencer inhibits expression of a heterologous promoter and that this repression requires an intact YY1 binding site. We have also demonstrated that GATA proteins activate expression of e-globin in erythroid cells but in a non-erythroid cell type represses expression. These data suggest that the mechanism of silencing of the e-globin gene in adult erythroid cells may involve unusual transcriptional repression by both GATA and YY1 proteins, probably mediated by one or more other transcriptional factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 25077-05 LCB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Globin Gene Expression and the Treatment of Hemoglobinopathies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Schu-zen Huang	Senior Staff Fellow	LCB, NIDDK
Others:	Griffin P. Rodgers	Chief, Molecular Hematology Section	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK
	Mai-Jue Chen and Jun Sun	Special Volunteers	LCB, NIDDK

COOPERATING UNITS (if any)
Shanghai Institute of Medical Genetics and Children's Hospital, Shanhai, China (Dr. Y.-T. Zeng)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL MAN-YEARS: 1.4	PROFESSIONAL: 1.4	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hemoglobin production in humans is characterized by two major switches, from embryonic to fetal, during the transition from yolk sax to the liver stage of hematopoiesis and from fetal to adult hemoglobin around the perinatal period. Any disturbance in these switching controls may result in abnormalities of globin gene expression and hemoglobin disorders (i.e., thalassemia, HPFH). The specific pattern of globin gene expression during development has been characterized. It has been generally assumed that the switches from embryonic to fetal globin protein synthesis accurately reflects a corresponding switch in mRNA, and the lack of embryonic globin in adult life ins due to a complete turn off of embryonic gene transcription. Recently, we used a two-phase liquid human adult erythroid (HAE) cell culture procedure, coupled with RT-PCR mRNA quantitative method, to set up a new system, for characterizing the pattern of embryonic epsilon globin gene expression at the mRNA level. Based on the trial experiments in RNA's from normal individuals, K562 cells and the mix RNA of normal individual + K562 cell, primers for specific amplification of epsilon gene were designed and screened so that the whole three exon sequences could be amplified with a high sensitivity, accuracy and reproducibility. We found, unexpectedly, that; (1) The expression of epsilon gene was assessed at the level of steady-state mRNA in HAE cells; (2) With the cell life-age, the levels of gamma and beta mRNA were increased, peaking at day 5-7 and 11-13, respectively; while the ratio of gamma/beta mRNA was gradually reduced, from 1.03 at day 5 to 0.29 at day 11; (3) The epsilon mRNA was also present in a relatively abundant amount in adult peripheral blood erythroid cells. The expression of epsilon mRNA in adult reticulocytes and HAE cells appears to be erythroid specific, as it was not detected in nonerythroid hematopoietic cells (B-cell Raji, T-cell Jurkat). In general, these data appear to be in accordance with the timed changes of globin gene expression during development. The result also indicates a significant discrepancy between the embryonic epsilon mRNA and the absence of the epsilon globin chain in adult blood. The presence of embryonic epsilon mRNA in adults raises the possibility that basal expression may increase in response to erythroid stress in a fashion similar to that seen in fetal hemoglobin. Therefore, the methodology we developed provides a powerful tool for further studies of globin gene switches and the function of the epsilon globin silencer, as well as the mechanism of HU effects on globin gene expression. Current work is in progress aimed at studying the action of pharmacological induction of the expression of gamma globin gene, and possibly epsilon gene using this newly developed methodology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 25078-04 LCB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Globin Expression in an Erythroid Progenitor Culture System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Eitan Fibach	Visiting Associate	LCB, NIDDK
Others:	Griffin P. Rodgers	Chief, Molecular Hematology Section	LCB, NIDDK
	Constance T. Noguchi	Chief, Molecular Cell Biology Section	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS (if any)
Clinical Pharmacology Branch - NCI (Dr. Dvorit Samid)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION NIDDK, Bethesda, MD 20892-1822

TOTAL MAN-YEARS: 1.3	PROFESSIONAL: 1.3	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Increased fetal hemoglobin (HbF) in cells of patients with beta hemoglobinopathies (thalassemia and sickle cell anemia) ameliorates the clinical symptoms of the underlying disease. Recently, several pharmacologic agents have been used to stimulate HbF synthesis; treatment of patients with 5-azacytidine or hydroxyurea resulted in higher HbF levels; yet, the clinical benefit is still unclear. Since these agents are either toxic and/or have been implicated in carcinogenesis, there is a considerable interest in other, less toxic agents with the potential to increase HbF. So far only a handful of agents have been tested, mainly due to the lack of an appropriate experimental system that allows a rapid and accurate determination of the effect on relevant cells.

We have recently developed a novel, two-phase liquid culture system for growing erythroid progenitors derived from the peripheral blood of normal individuals and patients with hemoglobinopathies. We showed that the system recapitulates many hematological effects of hydroxyurea in vivo, including stimulation of HbF production. The purpose of our research is to utilize this procedure and modified methods for quantitation of hemoglobins developed by us to study the regulation of Hb production and to screen agents for their HbF-stimulating potential with the final objective to produce an efficient, low toxicity therapy. The experiments include optimization of the conditions for stimulation of HbF production, followed by screening of various agents (including derivatives of hydroxyurea, phenyl-fatty acids, erythropoietin, hemin) and subsequently studying their mode(s) of action. The latter studies will provide the rationale for further screening of additional agents and for their chemical modification in order to increase efficacy. Classification of the drugs according to functional and mechanistic considerations will enhance experiments with combination of drugs belonging to different groups and, thus, expected to act synergistically.

In addition, we are attempting to develop, based on the cell culture system, an assay for evaluating an individual patient's response to a particular drug or drug combination. This will prevent both expensive and potentially risky treatment from patients which do no respond and suggest an alternative treatment (e.g. by other agents).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 25079-03 LCB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Filterability of Mixtures of Sickle and Normal Erythrocytes - Implication for Exchange Transfusion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Setsuo Hasegawa	Special Volunteer	LCB, NIDDK
Others:	Griffin P. Rodgers	Chief, Molecular Hematology Section	LCB, NIDDK
	Constance T. Noguchi	Chief, Molecular Cell Biology Section	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS (if any)
Nippon Medical School, Tokyo, Japan (N. Uyesaka)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL MAN-YEARS: 1.3	PROFESSIONAL: 1.3	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

RBC transfusion has long been and currently remains an integral part of the management of sickle cell disease(SCD). The judicious use of blood can be both life-saving and life-prolonging in a variety of clinical settings. There are two broad indications for blood transfusion in SCD, maintenance of the oxygen-carrying capacity of the blood and dilution of the circulating HbS-containing cells. The latter may be accomplished both by the direct dilutional effect of transfused blood but also secondarily by suppression of the bone marrow and decreased production of cells capable of sickling.

Erythrocyte exchange offers numerous potential advantages over simple transfusion for the management of certain complications of SCD, especially if there is concern of causing circulatory overload or inducing a hyperviscosity state, which may actually exacerbate the propensity to sickle by retarding capillary transit. Guidelines for exchange transfusion therapy should be derived from an understanding of the rheological behavior of sickle cells. Microvascular occlusion by poorly deformable erythrocytes is believed to be the key pathophysiologic event in SCD. We, therefore, investigated the deformability of sickle(SS) cells and their mixture with normal(AA) cells in terms of filterability through nickel mesh. We also examined the role of dense SS cells and dense SS reticulocytes in overall deformability. In this study, we found that the filtration of mixtures of SS and AA erythrocytes was affected in almost linear fashion by both the %SS erythrocytes and %dense cells, and that dense cells impaired the filtration of these mixtures about 25 times as much as that of SS erythrocytes with no dense cells did. Filtration of mixtures of SS cells with no dense cells and AA cells of more than 60% was almost the same as that of normal erythrocytes. These results suggest that exchange transfusion therapy should aim at decreasing dense cells as much as possible, as well as at keeping the level of SS cells to the total cells below 40%. Our results also indicate the possibility that dense SS reticulocytes involved in reduced deformability. Our data, using an automated reticulocyte analyzer, showed that the proportion of high fluorescence-reticulocytes was elevated in the patients with SCD, which indicated the increased regeneration of immature reticulocytes. The benefit of exchange transfusion, therefore, can be not only due to direct dilution effect, but also due to suppression of SS reticulocytes. These studies can provide the practical guideline for exchange transfusion program based on the rheological behavior of sickle cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25080-03 LCB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation Of Erythropoietin Receptor Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Chun Liu	Visiting Fellow	LCB, NIDDK
Others:	Constance T. Noguchi	Chief, Molecular Cell Biology Section	LCB, NIDDK

COOPERATING UNITS (if any)
LMGD, NICHD (Dr. Kathleen Mahan)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Cell Biology

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL MAN-YEARS: 1.1	PROFESSIONAL: 1.1	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The hormone erythropoietin (EPO), which interacts with erythropoietin receptor (EPOR), a high affinity cell surface receptor on the developing erythroblasts is critical for normal erythroid development. Intracellular signaling following binding of the ligand to its receptor results in a proliferative response and ensures differentiation of the erythroid lineage. EPO may have other functions too. EPO-R has been detected in placenta and endothelial cells. EPO was also shown to stimulate the production of immunoglobins in B-cells. Recent data in this laboratory indicated that EPOR is expressed in developing brain of the mouse embryo.

A 15kb Genomic DNA of the human EPOR has been cloned in this laboratory and two larger human genomic clones containing 85kb fragments were recently isolated. We are using these to study the expression and transcriptional regulation of the EPO-R gene. Preliminary result of in situ hybridization using a RNA probe generated by in vitro transcription of EPOR cDNA to nine day mouse embryo has shown staining in the head and back area and a streak along the center of the body. These structures are yet to be determined. Transient transfection of eukaryotic cell line is also being used to identify upstream cis-regulatory elements and their regulation by transcription factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25082-03 LCB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Human Erythropoietin Receptor Transcription

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kyung Chin	Microbiologist	LCB, NIDDK
Others:	Constance T. Noguchi	Chief, Molecular Cell Biology Section	LCB, NIDDK

COOPERATING UNITS (if any)
Brown University (Dr. A.A. Anagnostou)
Cornell University (Dr. B. Weksler)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Cell Biology

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL MAN-YEARS: 1.2	PROFESSIONAL: 0.2	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The expression of the erythropoietin receptor (EpoR) on the surface of erythroid progenitors is necessary for the binding of erythropoietin (Epo), the primary stimulator of erythropoiesis. We examined control elements within the promoter of the human erythropoietin receptor (hEpoR) gene which contains binding sites for transcription factors, GATA-1 with erythroid specificity and Sp1, but no TATA sequences. In the hEpoR proximal promoter region we have observed binding of nuclear proteins to the GATA-1, Sp1 and AP2 binding consensus sequences, and a region around -175 bp. Regions containing the AP2 binding site and around -175 bp provided positive and negative transcription regulation, respectively. The GATA-1 binding site contributed to positive regulation in erythroid cells only. Although the endogenous levels of GATA-1 in K562 and OCIM1 cells were comparable, cotransfection of the hEpoR promoter with a GATA-1 expression vector to further increase GATA-1 levels increased hEpoR promoter activity in K562 cells reaching a five fold increase at saturation, but exhibited no effect in OCIM1 cells. In non-erythroid cells, hEpoR promoter activity was increased by co-transfection with GATA-1 and to a lesser extent by mutation and inversion of the GATA-1 binding site. GATA-1 provides negative regulation of the hEpoR promoter in non-erythroid cells. Promoter activity was still present with deletion of the GATA-1 binding site leaving the Sp1 binding site. Although mutation of the Sp1 binding site markedly decreased promoter activity, the promoter could still be transactivated by GATA-1. Hence, as with other TATA-less promoters, Sp1 alone can activate transactivate, indicating that Sp1 is not required and may not interact directly with GATA-1 for hEpoR transactivation. The role of the Sp1 and GATA-1 binding sites in the hEpoR promoter suggest that interactions of Sp1 and GATA-1 with the hEpoR gene contribute significantly to the differential transcription observed for hEpoR tissue and stage specific expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25083-02 LCB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Utilization of Triple Helical DNA as a Potential Means of Increasing Gamma Globin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Alphonso Brown	Howard Hughes Scholar	LCB, NIDDK
Others:	Griffin P. Rodgers	Chief, Molecular Hemotology Unit	LCB, NIDDK
	Eitan Fibach	Visiting Associate	LCB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS: PROFESSIONAL: OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been suspended.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25084-01 LCB

PERIOD COVERED
October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
A Transgenic Pig Model For Sickle Cell Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Steven G. Shapiro	Special Volunteer	LCB, NIDDK
Others:	Alan N. Schechter	Chief	LCB, NIDDK
	Constance Noguchi	Section Chief	LCB, NIDDK
	Griffin P. Rodgers	Section Chief	LCB, NIDDK

COOPERATING UNITS (if any)
Veterinary Resources Program, NCRR (Victoria Hampshire, VMD, Chief, Carnivore and Ungulate Unit, Bethesda, Md.; Axel Wolff, DVM, Chief, Ungulate Unit, Poolesville, Md.)

LAB/BRANCH Laboratory of Chemical Biology

SECTION

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892-1822

TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.5	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have begun to make a transgenic pig model for sickle cell disease (SS disease) to facilitate studies for sickle cell pathology and evaluations of new drug and gene therapies. Previous attempts to make trangenic models using the mouse have failed to produce the clinical symptomatology of SS disease, in spite of the production, in some cases, of significant amounts of human sickle hemoglobin (HbS) and evidence for intracellular polymerization of hemoglobin. The failure of the transgenic mice to develop significant sickle cell disease pathology appears to be due to differences in the vascular anatomy and physiology between mice and humans. On the other hand, the pig provides an excellent model for human circulatory physiology, significantly, for our purposes, evincing cognate responses to vaso-active compounds, similarities in capillary bed density, cerebrovascular diseases and certain red cell structural properties. The technology to produce transgenic farm (full-size) pigs is also well established.

To produce transgenic animals with high level globin transgene expression, a genetic regulatory region called the β-globin locus control region (LCR), usually located upstream of the β-globin gene cluster, has been shown to be required in cis. We have prepared DNA constructions containing the functional portions of the human β-globin LCR (mini-LCR) linked to the human α-2-globin gene or a modified human sickle β-globin gene with additional mutations to promote polymerization (βSAD, containing additional mutations Antilles and D Punjab) for microinjection into the pigs. At the same time, to facilitate the maintenance and clinical analysis of the transgenic pigs we have begun to develop the NIH-minipig as a transgenic system. Using a regimen of purified FSH, significant superovulation has been obtained (40 eggs/pig). Following breeding, fertilized eggs have been recovered and procedures for microinjection, surgical reimplantation and maintenance of pregnancy are being developed to extend the transgenic technology to this more conveniently-sized pig variety. Once established, the transgenic pig model that produces high levels of human sickle hemoglobin and demonstrates SS disease pathology, should greatly accelerate our efforts to understand and treat the disease.

U.S. GOVERNMENT PRINTING OFFICE: 1991

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29001-22-LCP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular dynamics and vibrational characteristics of membrane assemblies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Ira W. Levin	Research Chemist	LCP-NIDDK
Others:	E. Neil Lewis	Visiting Associate	LCP-NIDDK
	Tonya Herne	IRTA	LCP-NIDDK
	Curtis Meuse	IRTA	LCP-NIDDK
	Steven Barnett	IRTA	LCP-NIDDK

COOPERATING UNITS *(if any)*

C. Huang, School of Medicine, Univ. of VA; J.S. Vincent, Univ. of MD; R. Hendler, NHLBI

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. We have continued the pioneering of *vibrational spectroscopic imaging* microscopy techniques by advancing the methodology to *near-infrared absorption imaging* using state-of-the-art indium antimonide (InSb) focal plane array detection. Since near-infrared spectroscopy is a sensitive, non-invasive method used extensively in chemical analyses, its integration with imaging technologies represents a potent tool for the study of a wide range of materials. In particular, a 128x128 pixel InSb staring array (multichannel) imaging detector has been combined with a refractive optical microscope and an acousto-optic tunable filter (AOTF) to display chemically discriminated, spatially resolved, vibrational spectroscopic images of biological and polymeric systems. AOTFs are computer controlled bandpass filters that provide high speed, random wavelength access, wide spectral coverage and moderate spectral resolution. Although AOTFs inherently have a wide range of spectroscopic applications, we have first applied this particular technology to near-infrared absorption microscopy between 1-2.5 μm. This spectral interval is well matched to the optical characteristics of both the near-infrared refractive microscope and the AOTF, thereby providing near-diffraction limited performance with a practical spatial resolution of 1-2 μm.

2. Since mid-infrared spectroscopy (2.5 μm to 25 μm or 4,000-400 cm^{-1}) provides a wealth of spectral probes for analyses of materials extending from the biochemical and biophysical disciplines to the analytical chemistry arena, we have adapted our absorption imaging microscopy techniques to this spectral interval.

   A. A two-dimensional indium antimonide detector has been modified to serve in the 4,000-1,800 cm^{-1} region. A system was built using dielectric filters for passing infrared radiation in the appropriate spectral region and infrared cassegrain optics for the microscope assembly. Domain structures of model lipid systems and of tissue slices of a monkey cerebellum were examined.

   B. The ability to obtain mid-infrared vibrational spectroscopic images was dramatically extended by our adaption of an infrared *step-scan* interferometer coupled to a microscope and focal plane array detector. Spatial resolution is of the order of 5-7 μm, while the spectral resolution can vary from 8-16 cm^{-1}. In this application, approximately 16,000 spectra, which constitute the image comprised of 128x128 pixels, are recorded in ~12 seconds. This is the first time this mid-infrared imaging technique has been demonstrated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-DK-29005-20-LCP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Asymmetric Synthesis: Structure, Stereochemistry, and NMR

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.'s	Herman Ziffer	Research Chemist	LCP/NIDDK
Others:	Yuming Pu	Visiting Fellow (2 months)	LCP/NIDDK
	Daniel S. Torok	IRTA Fellow	LCS/NIMH

COOPERATING UNITS (if any)

Dr. Sanford Markey, LCS/NIMH; Dr. H. J. C. Yeh, LAC/NIDDK
Prof. Steven R. Meshnick, Dept. of Epidemiology, University of Michigan School of Public Health

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.18	1.18	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In earlier work we developed a method for preparing 14-[^{2}H]-arteether and have now employed that reaction sequence to prepare 14-[^{3}H]-arteether. The ^{3}H labeled arteether was used to study the mechanism of action of this group of antimalarial drugs. Infected and uninfected red blood cells were treated with labeled arteether, dihydroartemisinin and a third drug that also contains the same endoperoxide grouping. The same six proteins were found to react in infected cells, whereas none of the proteins in the uninfected red cells reacted with these compounds nor with a control, [^{3}H]- desoxyarteether. These findings make it possible to identify, purify and study the structure and function of the reactive proteins.

Diastereofacial additions of primary alcohols to the carbon-carbon double bond of anhydrodihydroartemisinin, catalyzed by triphenyl-phosphine hydrobromide, were employed to prepare a series of 11-epidihydroartemisinin derivatives. These additions enabled us to prepare previously unknown epimeric arteethers. The antimalarial activity of 11-epi-dihydroartemisinin was examined and found to be half as active as the isomer with the natural configuration at C-11. A study of minor reaction products as a function of the number of equivalents of alcohol employed, provided invaluable information on the mechanism of the reaction and on the stereochemistry of the reaction using Polarized π-Frontier Molecular Orbital Theory.

Our study of the regio- and stereo-selectivity of *Beauveria sulfurescens* mediated hydroxylation of 1,4-disubstituted benzenes have shown that the β-carbon of the alkyl side chain is selectively hydroxylated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29008-23-LCP

PERIOD COVERED

October 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Electric and molecular structure investigation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Ruth McDiarmid	Research Chemist	LCP-NIDDK
Others:	Xing Xing	Visiting Fellow	LCP-NIDDK

COOPERATING UNITS *(if any)*

Leo Klasinc and Branka Kovac, Rudjer Boskovic Institute, Zagreb, Croatia
Bjoern Roos, Chem. Centre, Lund, Sweden

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Spectroscopy and Structure

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Optical absorption and polarization-selected 2 photon resonant multiphoton ionization (REMPI) and photoacoustic (PA) spectroscopic measurements on static samples and REMPI measurements on jet cooled samples of norbornadiene have been carried out to interpret the spectrum of this molecule, compare the results obtained by the different techniques, and assess theoretical calculations of the energies and equilibrium geometries of the excited states of the molecule. All three methods detected the structured 3s Rydberg <-X transition of norbornadiene but only the optical measurement could directly detect either of two lower energy diffuse valence transitions of this molecule. Detailed comparisons of the REMPI and PA spectra of the 3s Rydberg/second valence region of the spectrum demonstrated that only the 3s Rydberg transition is directly observable by REMPI spectroscopy. Once excited the 3s Rydberg transition of the molecule rapidly transfers energy to the valence state which then decays rapidly to the ground state by the emission of heat. The excess energy dependence of the decay rate of the 3s Rydberg state was determined. From these results we hypothesize that the valence transition has a small 2 photon cross section. The vibrational substructure of the 3s Rydberg <-X transition of norbornadiene was also analyzed to determine its excited state geometry to the greatest extent possible.

The far UV spectrum of acetone was studied by 3-photon REMPI and PA spectroscopies on static and jet-cooled samples. Two of the observed transition were deduced to be coupled by an asymetric vibration of the molecule. The analysis of this spectrum is continuing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29010-22-LCP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Dynamics of Proteins and Studies on Sickle Cell Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	William A. Eaton	Medical Officer	LCP-NIDDK
Others:	James Hofrichter	Research Chemist	LCP-NIDDK
	Eric Henry	Research Physicist	LCP-NIDDK
	Garrott Christoph	Physicist	LCP-NIDDK
	Steve Hagen	Staff Fellow	LCP-NIDDK
	Chi-Kin Chan	Visiting Associate	LCP-NIDDK
	Yi Hu	Visiting Associate	LCP-NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Macromolecular Biophysics

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Time resolved optical spectroscopy in photodissociation experiments and molecular dynamics simulations are being used to investigate protein folding and structure function relations in heme proteins. Fast events in the folding of cytochrome *c* are being studied by optically triggering the folding reaction with nanosecond laser pulses. Before folding begins transient binding of both non-native and native ligands from the unfolded polypeptide has been observed on a microsecond time scale.

We have confirmed the prediction from our previous studies on the viscosity dependence of the conformational relaxation in myoglobin that the low temperature behavior of myoglobin with a "frozen" distribution of conformational substates can be mimicked in a room temperature glass, including the observation of a wide distribution of geminate rebinding rates and spectral changes caused by kinetic hole burning.

Extensive modelling of the geminate rebinding and conformational relaxation kinetics of human hemoglobin show that a satisfactory explanation of a very demanding set of data can be explained by a two-state allosteric model.

A laser-photolysis light-scattering technique has been used to monitor intracellular hemoglobin S polymerization in sickle cell disease patients before and after treatment with hydroxyurea. Hydroxyurea therapy leads to a reduction in the fraction of rapidly sickling cells, and an increase in the fraction of non-sickling cells expected from the increase in F cells. The median delay time, however, is still smaller than that observed in SC patients, suggesting that a major therapeutic effect from hydroxyurea should not be expected.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29011-23-LCP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The physics and chemistry of photoreception

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.	William A. Hagins	Medical Officer	LCP-NIDDK
Others:	S. Yoshikami	Research Biologists	LCP-NIDDK
	P. Ross	Research Chemist	LMP-NIDDK
	Mark Vivino	Computer Systems Analyst	CSL-DCRT

COOPERATING UNITS *(if any)*

Sudhir Sabu, Student Volunteer, Thomas Jefferson High School, Alexandria, VA, Ramin Rashidian, Student Volunteer, Murray State University, Murray, KY 42071.

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Membrane Biophysics

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Calcium metabolism in retinal rod outer segments during phototransduction is being studied with the aid of fluorochromic dyes introduced into retinas by new methods and studied by quantitative microscopic image systems.

Calorimetric, X-ray microanalytic and photometric methods are being used to study ionic and biochemical events during phototransduction.

New techniques for breaching the blood-brain barrier to introduce polar molecules into brain and retinal tissue are under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29016-17-LCP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Macromolecular dynamics and assembly reactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	James Hofrichter	Research Chemist	LCP-NIDDK
Others:	Garrott Christoph	Physicist	LCP-NIDDK
	Eric Henry	Research Chemist	LCP-NIDDK
	William A. Eaton	Medical Officer	LCP-NIDDK
	Steve Hagen	Staff Fellow	LCP-NIDDK
	Chi-Kin Chan	Visiting Associate	LCP-NIDDK
	Yi Hu	Visiting Associate	LCP-NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Laser Biophysics and Spectroscopy

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Time-resolved absorption spectroscopy is used to study the dynamics of protein structural changes subsequent to excitation with short laser pulses. Molecular models for the protein dynamics are used to fit and interpret the measured data.

A. The kinetics of ligand binding and conformational changes for sperm whale myoglobin in sugar glasses have been studied at temperatures from 100K to 300 K following the photodissociation of carbon monoxide from the hemes. The results confirm the prediction, based on work of Ansari et al. (1992,1994) that conformational relaxation can be inhibited by increasing the solvent viscosity.

B. The dynamics of short-lived states on the folding pathway of cytochrome c have been investigated using time-resolved absorption spectroscopy. The binding of carbon monoxide destabilized the folded state of reduced cytochrome c, permitting folding to be initiated by photodissociation of this ligand. We have observed transient binding of other at least two different side chains to the heme subsequent to photodissociation of CO.

C. The kinetics of intracellular polymerization of hemoglobin S have been studied for cells from patients undergoing treatment with hydroxyurea. The delay times increased dramatically in the course of treatment, with the median value increased by a factor of 5 after 12 weeks of treatment and more than a factor of 10 after 24 weeks.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-DK-29019-14-LCP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Theoretical studies on the dynamical aspects of macromolecular function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	A. Szabo	Research Chemist	LCP-NIDDK
Others:	X. Zhou	Visiting Associate	LCP-NIDDK

COOPERATING UNITS *(if any)*

Eaton W.A., and Henry E., - (all) LCP-NIDDK; Freed K., Chicago; Perico A., Genova; Austin R., Princeton; Douglas J.F., and Hubbard J., (NIST).

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Theoretical Biophysical Chemistry Section

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The kinetics of the geminate rebinding of nitric oxide following photodissociation to myoglobin was simulated using the technique of molecular dynamics. The kinetic progress curve, obtained by averaging 100 trajectories, could be well reproduced by a simple model of a Langevin particle moving in a potential of mean force calculated from nonreactive trajectories. The rate of escape from the energy well adjacent to the heme was in good agreement with the value calculated from experimental data, suggesting that a multiple-well model provides a plausible explanation for the nonexponential rebinding kinetics. A Brownian dynamics algorithm, originally devised for calculating diffusion-controlled reaction rates, has been adapted to calculate the transalational hydrodynamic friction (i.e., the reciprocal of the diffusion coefficient) of objects with complex shapes. This work in effect exploits a deep analogy between two seemingly unrelated physical quantities, and leads to a novel and highly efficient way of calculating the diffusion coefficients of macromolecules. A theory has been developed for the electrophoresis of DNA molecules in microlithographically constructed synthetic gels consisting of an essentially two dimensional regular array of posts. Specifically, the average "unhooking" time for a DNA molecule that got hooked on a post to form a U-shaped structure was calculated. Good agreement was found between theory and experiment, indicating that one fundamental aspect of the mechanism of length fractionation can be fully understood on a microscopic level. An efficient algorithm developed in this laboratory to calculate the electrostatic properties of macromolecules has been applied to the cytochrome c-cytochrome peroxidase system to elucidate the influence of electrostatic interactions on redox potentials, the free energy of complex formation and the efficiency of electron transfer. In addition to successfully predicting the effect of mutations on those properties, these calculations revealed the structural basis of how the redox potential and the rate of election transfer is controlled by the protein environment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29020-09-LCP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nuclear magnetic resonance: new methods and molecular structure determination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Ad Bax Visiting Scientist LCP-NIDDK

Others: Tschudin, Delaglio, Wang, Vuister, Grzesiek, Live
Zhu (Special Volunteer)
Richard Do (Summer Student)
Kuboniwa (Special Volunteer)
John Marquardt (Special Volunteer)

COOPERATING UNITS *(if any)*

Marius Clore & Angela Gronenborn, LCP-NIDDK; Dennis Torchia, BRB-NIDR; Claude Klee & Carl Wu, LB-NCI

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Biophysical Nuclear Magnetic Resonance Section

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.5	7.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

New methods have been developed which enhance the sensitivity of protein NMR by transferring magnetization from the H_2O solvent to the protein. Pulsed field gradients and selective pulses are used to ensure that the water magnetization remains close to its equilibrium value during the entire experiment. Cross relaxation and exchange of labile protein protons with solvent then result in increased protein signal intensity. The enhancement ranges from 10-50% for most experiments that are conducted in H_2O solution, to a three-fold enhancement when studying the interaction between solvent and protein. This new methodology was used to prove that the HIV protease inhibitor DMP-323 indeed displaces the conserved water molecule observed in other complexes between inhibitors and the protease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29021-09-LCP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Conformation and dynamics of biological molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Eric Henry	Research Physicist	LCP-NIDDK
Others:	William Eaton	Medical Officer	LCP-NIDDK
	Colleen Jones	Staff Fellow	LCP-NIDDK
	James Hofrichter	Research Chemist	LCP-NIDDK

COOPERATING UNITS *(if any)*

Stuart J. Edelstein	University of Geneva
Olivier Schaad	University of Geneva

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Biophysical Chemistry Section

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have used kinetic modelling to analyze sets of time-resolved spectroscopic data measured for hemoglobin over a range of fractional ligand photodissociation. The model used is a kinetic formulation of the two-state allosteric model extended in a minimal fashion to incorporate the geminate ligand rebinding and tertiary conformational changes detected in the measurements. The model incorporates three spectroscopically distinguishable states of unliganded hemoglobin subunits, and the kinetic description includes a stretched-exponential form for the tertiary conformational relaxation as well as a linear free energy relation connecting the quaternary transition rates for tetramers with different numbers of bound ligands. Fits using this model produce spectra for the three unliganded species which resemble the spectrum of deoxyhemoglobin and differ in a manner that is consistent with the ligand binding affinities of the species. The fits predict that both tertiary and quaternary conformational relaxations have a marked effect on the geminate ligand rebinding rate. We have also shown that a four-state allosteric model provides a common mechanistic description of the kinetics of neurotransmitter binding, channel opening and desensitization of various synaptic ionotropic receptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29022-06-LCP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Studies of AIDS proteins by NMR

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.'S	Angela M. Gronenborn	Visiting Scientist	LCP-NIDDK
	G. Marius Clore	Visiting Scientist	LCP-NIDDK
	Ad Bax	Visiting Scientist	LCP-NIDDK
Others	Robert Clubb	Guest Researcher	LCP-NIDDK
	Daniel Garrett	IRTA Fellow	LCP-NIDDK
	Patricia Lodi	Guest Researcher	LCP-NIDDK
	Jim Omichinski	Staff Fellow	LCP-NIDDK

COOPERATING UNITS *(if any)*

NCI (Ettore Appella, K. Sakaguchi, A. Wlodawer); NCRR (Marc Lewis); NHLBI (Warren Leonard); Protein Expression Laboratory (Paul Wingfield); Cornell (Andy Karplus); Oxford (Chris Dobson).

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Sections on Structural Biology, Protein NMR Spectroscopy, and Biophysical NMR

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.6	2.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work has been carried out on a number of structural problems related to proteins derived from the HIV virus. These include the p7 nucleocapsid protein and nef of HIV-1, and proteins of the immune system, in particular human macrophage inflammatory protein-1β, interleukin-8 and interleukin-4. The RNA binding properties of the p7 nucleocapsid protein have been investigated and structural studies of a specific p7-RNA complex have been initiated. In addition, work on the complex of the transcription factor GATA-1 with its cognate DNA target site has been pursued to identify and localize bound water.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29023-04-LCP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Determination of Three-Dimensional Structures of Macromolecules in Solution by NMR

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	G. Marius Clore	Visiting Scientist	LCP-NIDDK
	Angela M. Gronenborn	Visiting Scientist	LCP-NIDDK
Other:	Jim Omichinski	Staff Fellow	LCP-NIDDK
	John Kuszewski	Pre-Doctoral IRTA	LCP-NIDDK
	Terersa Strzelecka	Visiting Associate	LCP-NIDDK
	Patricia Lodi and Robert Clubb	Guest Researchers	LCP-NIDDK
	Jeff Huth, Jun Qin, Milton Werner, William Kennedy, and Daniel Garrett	IRTA Fellows	LCP-NIDDK

COOPERATING UNITS *(if any)*

Lab. Chemical Physics, NIDDK (A. Bax) Lab. of Molecular Biology, NIDDK (G. Felsenfeld, K. Mizuuchi); NCI (E. Appella, D. Covell, J. Barchi, A. Wlodawer); NHLBI (W. Leonard); NIMH (H. Nash); Yale (K. Williams); C. Dobson (U. of Oxford).

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section of Protein NMR Spectroscopy and Section of Structural Biology

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.8	4.8	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work in this laboratory has been focussed on the determination of three-dimensional structures of larger proteins in solution by NMR, with a particular emphasis on cytokines, immune related proteins, DNA-protein complexes, and protein-protein complexes. A considerable effort has been placed on the development of three- and four-dimensional heteronuclear NMR to extend the application of NMR as a method for determining three-dimensional structures of proteins in solution beyond the limits of conventional two-dimensional NMR (~100 residues) to molecules in the 150- to 400-residue range.

High resolution solution structures of a number proteins have been determined. These include the oligomerization domain of p53, the β chemokine macrophage inflammatory protein-1β (hMIP-1β), oxidized and reduced human thioredoxin, and the trypsin inhibitor of Ascaris. Extensive use in these studies has been made of multi-dimensional heteronuclear NMR and of systematic conformational searches to obtain stereospecific assignments and torsion angle restraints which have enabled us to obtain very high resolution structures comparable in accuracy to 2 Å resolution X-ray structures. The typical accuracy attainable is 0.2-0.4 Å for the backbone atoms and 0.3-0.5 Å for the internal side chains.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29025-05-LCP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Investigations of Macromolecular Structures and Dynamics in Solution by NMR

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.'S	Angela M. Gronenborn	Visiting Scientist	LCP-NIDDK
	G. Marius Clore	Visiting Scientist	LCP-NIDDK
Others:	John Kuszewski	Pre-Doctoral IRTA	LCP-NIDDK
	Teresa Strzelecka	Visiting Associate	LCP-NIDDK
	Jim Omichinski	Staff Fellow	LCP-NIDDK
	Patricia Lodi and Robert Clubb	Guest Researchers	LCP-NIDDK
	Milton Werner, Jun Qin, Daniel Garrett, Jeff Huth, and William Kennedy	IRTA Fellows	LCP-NIDDK

COOPERATING UNITS *(if any)*

LCP, NIDDK (Ad Bax); LMB, NIDDK (Gary Felsenfeld, Kyoshi Mizuuchi); NCI (Ettore Appella, Dave Covell, Joe Barchi, Alex Wlodawer); NHLBI (Warren Leonard); NIMH (Howard Nash); Yale (Ken Williamson); Oxford (Chris Dobson).

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section of Structural Biology and Section of Protein Nuclear Magnetic Resonance

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.8	4.8	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of the overall research in this laboratory is centered on achieving as complete a description as possible for the structures of peptides, proteins, nucleic acids and their complexes in solution, principally by NMR spectroscopy. At present particular emphasis is being placed on developing approaches which allow the investigation of larger and complex systems as well as increase the precision with which these solution structures can be obtained, studies aimed at correlating structure and function, and experiments aimed at investigating protein folding.

Structures for several proteins have been determined and analyzed. These include oxidized and reduced human thioredoxin, the oligometization domain of p53, the cytokine hMIP-1β, and the Ascaris trypsin inhibitor. These studies have exploited many novel 3D and 4D heteronuclear NMR experiments to dramatically increase spectral resolution and thereby resolve assignment ambiguities in larger proteins. The site of interaction of interleukin-8 with the N-terminal cytoplasmic portion of its cell surface receptor has been identified, and the dynamics of the IgG binding domain of Streptococcal G have been investigated. Finally, the folding pathway and kinetics of the highly stable small IgG binding domain of Streptococcal protein G has been studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29026-06-LCP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

NMR and other spectroscopic studies of molecular structure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Edwin D. Becker	Research Chemist	LCP-NIDDK
Others:	C.L. Khetrapal	Special Volunteer	LCP-NIDDK

COOPERATING UNITS *(if any)*

Molecular Graphics and Simulation Laboratory, DCRT Sophisticated Instruments Facility, Indian Institute of Science, Bangalore, India Chemistry Department, Rudger Boskovic Institute, Zagreb, Croatia

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Nuclear Magnetic Resonance

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

NMR investigations with liquid crystals are being used to study weak molecular interactions and to provide information on the degree of ordering of molecules dissolved in a liquid crystal solvent. A sample of chloroform enriched with both carbon-13 and deuterium has been studied in a nematic solvent while being spun at the "magic angle." The relative intensities of the spinning sidebands have been shown to provide information on the sign of the order parameter.

We are continuing to explore the mechanism and potential applications of the effect of isotopic substitution on NMR chemical shifts of nuclei that are several bonds away from the site of substitution. Studies of a number of differently substituted binuclear aromatic molecules (including cis- and trans-stilbenes, trans-azobenzene, diphenyl-acetylene and diphenylethane) were previously reported to show isotope-induced changes in carbon-13 chemical shifts through as many as 10 chemical bonds and as small as 0.64 parts per billion. The relation of the observations to details of molecular structure is being evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-DK-29027-06-LCP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Theoretical studies of dynamical processes in chemical physics and biophysics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Robert Zwanzig	Research Chemist	LCP-NIDDK

Others:

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Theoretical Biophysics Section

INSTITUTE AND LOCATION

NIH, NIDDK. Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several different projects are under way. (1) One is a continuing study of proposed theories of protein folding, and in particular, the problem of finding a theory that conforms to many computer simulations of folding. (2) Another is concerned with the "roughness" of a many body potential surface. Of particular importance is the distribution of extrema, especially saddle points, of the potential. (3) A third is a search for a good theory of the first contact of the ends of a long polymer undergoing Brownian motion. Extensive computer simulations have been performed; no current theory works.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29028-04-LCP

PERIOD COVERED

October 1993 through September 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Free energy conversion in biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Yi-der Chen	Research Chemist	LCP-NIDDK
Others:	Robert J. Rubin	Special Volunteer	LCP-NIDDK

COOPERATING UNITS *(If any)*

T. Y. Tsong, Dept. of Biochemistry, The Hong Kong University of Science and Technology, Hong Kong.

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Theoretical Biophysics Section

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The general aim of this project is to study the molecular mechanisms of free energy transduction in biology, such as in muscle contraction, active transport, flagellar rotation, etc. During this period, our work was concentrated on the continuing studies of fluctuation-induced active transports of ligands across membranes. It was suggested theoretically a few years ago that randomly fluctuating electric fields could be absorbed by charged ligand transporters and used to pump ligands across a membrane against a concentration gradient. In collaboration with Tsong and his colleagues we showed in this work that this prediction indeed could be verified experimentally using random telegraphy-type fluctuations. To provide a molecular description of the system, we developed a simple four-state transport model capable of simulating the measured data. We also investigated theoretically the general dynamic properties of a simplified two-state transport system under the influence of alternating rectangular (regularly oscillating) electric pulses, in relation to the general questions of how the fluctuating electric field and the ligand transport systems are coupled.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31100-29 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacologically Active Compounds from Amphibians and Other Natural Sources

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	J.W. Daly	Chief	LBC, NIDDK
Others:	C.R. Creveling	Research Chemist	LBC, NIDDK
	T. Spande	Research Chemist	LBC, NIDDK
	H.M. Garraffo	Visiting Associate	LBC, NIDDK
	K. Hutchinson	IRTA	LBC, NIDDK
	B. Badio	IRTA	LBC, NIDDK
	P. Jain	Visiting Fellow	LBC, NIDDK
	W. Padgett	Biologist	LBC, NIDDK

COOPERATING UNITS (if any)

T. Tokuyama, Osaka City U., Japan; C.W. Myers, AMNH, NYC; E. Gros, U. Buenos Aires, Argentina, J. Cover, NAIB, Baltimore, MD; M. Andriantsiferana, Madagascar; A.S. Rand, STRI, Panama City, Panama.

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Pharmacodynamics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.9	4.5	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Natural products provide a wide range of biologically active agents, many of which have unique profiles of pharmacological activity and therapeutic potential. Over three hundred alkaloids have been identified in extracts from amphibian skins. These include batrachotoxins, which are potent activators of sodium channels, the histrionicotoxins, which are noncompetitive blockers of nicotinic receptor channel complexes and potassium channels, and pumiliotoxins, which have myotonic and cardiotonic activity due to effects on sodium channels. Further alkaloids include 2,5-disubstituted decahydroquinolines, 3,5-disubstituted indolizidines, 5,8-disubstituted indolizidines, 1,4-disubstituted quinolizidines, 3,5-disubstituted pyrrolizidines, the pumiliotoxin-homopumiliotoxin-allopumiliotoxin class, and a variety of tricyclic alkaloids, including pyrrolizidine oximes, pseudophrynamines, cyclopentaquinolizidines, coccinellines, and the potent analgetic epibatidine. Most of these alkaloids have been detected and characterized from neotropical dendrobatid frogs. Pumiliotoxins also occur in one genus of Australian myobatrachid frogs, in one genus of South American bufonid toads and in one genus of Madagascan mantellid frogs. The myobatrachid frogs also contain a unique class of physostigmine-like alkaloids, the pseudophrynamines, while the bufonid toads and the mantellid frogs contain, in addition to pumiliotoxins, a wide array of quinolizidines, indolizidines, pyrrolizidines, decahydroquinolines and tricyclic alkaloids. Structures of several new alkaloids including a desoxypumiliotoxin, a trisubstituted indolizidine, a trimethyl azatricyclododecene, and a dimer derived apparently from an octahydroquinoline have been deduced. An FTIR method for distinguishing cis- and trans-disubstituted piperidines and pyrrolidines was developed. Synthetic efforts towards dehydropumiliotoxins, epibatidine analogs, pseudophrynamines and 1,4-disubstituted quinolizidines continue. Several alkaloids in dendrobatid frog skin have been shown to be derived from dietary arthropods via an extremely efficient alkaloid uptake system. Biologically active alkaloids have now been detected in two further genera of birds. Epibatidine was shown to owe its analgetic effects to potent nicotinic agonist activity. Remarkably, there is no enantioselectivity in this very potent alkaloid. N-Methylation did not affect activity. The des-chloro analog was fully active, providing a facile approach to radiolabelling. Further analogs are being investigated both as nicotinic agonists and as analgetics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31101-25 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacology and Metabolism of Biogenic Amines and Related Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: C. R. Creveling, Research Chemist, LBC, NIDDK
Others: None.

COOPERATING UNITS *(if any)*

Weisz, J., U. Penn., Hershey, PA; Inoue, K., Matsumoto Uni., Nagano, Japan; Moody, D., J. Hopkins Uni., Baltimore MD; Liehr, J., Uni. Texas, Galveston, TX; Winshilboum R., Mayo Clinic, Rochester, MN; T. Nagatsu, Fugita Health Uni.,

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Pharmacodynamics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The biochemistry, physiology, and pharmacology of biogenic amines, catecholsteroids, amino acid precursors and metabolic products, and various synthetic derivatives have been investigated. The general areas of study include the effects of fluorine substitution of the properties of biogenic amines, adrenergic antagonists and amino acids and catechol-O-methyltransferase (COMT). Studies on COMT include: 1) immunohistochemical localization and suppression of the induction of COMT in the luminal epithelium of pregnant and pseudopregnant rat following treatment with progesterone-receptor inhibitors including RU486 2) localization of COMT in the hamster kidney and in estrogen-induced carcinomas of hamster kidney 3) localization of COMT and COMT mRNA in normal human breast tissue 4) activity and localization of COMT and COMT mRNA in human endometrial and breast adenocarcinomas 5) the identification of monoamine oxidase A and B as one of the irreversible binding sites of procaine isothiocyanate 6) the identification and tissue distribution of 2-fluorourocanic acid in mouse in vivo; 6) development of microassay methods for the measurement of general anesthetics and their interaction with sodium channels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31102-23 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ion Channels, Receptors and Second Messengers in the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and

P.I.	J.W. Daly	Chief	LBC, NIDDK
Others:	B. Badio	IRTA	LBC, NIDDK
	Y. Shin	IRTA	LBC, NIDDK
	W. Padgett	Biologist	LBC, NIDDK

COOPERATING UNITS (if any)

T. Yasumoto, Tohoku Univ., Sendai, Japan; R.A. North, Vollum Institute, Portland, OR; F. Gusovsky, Eisai Res. Inst., Andover, MA.

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Pharmacodynamics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	1.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Calcium, sodium, potassium, and magnesium ions after translocation through ion channels or by transport proteins can cause activation of release processes, contractile proteins, adenylate and guanylate cyclase, phosphodiesterases, protein kinases, phospholipases, ATPases and other enzymes. Receptors of various types and various toxins serve to modulate ion channels and generation of second messengers including cyclic nucleotides, diacylglycerides, arachidonic acid and phosphatidic acid. Modulatory interactions or "cross-talk" occurs both between the second messenger systems and with the ion transport systems. Maitotoxin, a marine polyether increases phospholipid breakdown, leading to inositol phosphate and diacyclglyceride production. The primary site of action of maitotoxin appears to be a calcium channel. The maitotoxin-responses are effectively blocked by imidazoles that are also effective in blocking the so-called calcium-release-activated calcium (CRAC) channels, which are activated in plasma membranes of cells when IP_3-sensitive intracellular stores of calcium are depleted either through receptor-activation of phospholipase C or through blockade of intracellular Ca^{++}-ATPases by thapsigargin. However, other calcium blockers that prevent maitotoxin-responses do not block CRAC channels activated by ATP or fmLP in HL-60 cells. These include fluspirilene, penfluridol, loperamide and proadifen. The imidazoles (econazole, miconazole, SKF 96365, clotrimazole, calmidazolim) not only block maitotoxin-responses and CRAC channels, but also elicit *per se* an influx of calcium. Loperamide, while having no effect on $[Ca^{2+}]_i$ alone, appears to enhance CRAC channel-elicited influx. ATP activates phosphoinositide breakdown in HL-60 cells through a P_{2x}-receptor. In PC12 cells, ATP activates phosphoinositide breakdown through both P_{2x} and P_{2y}-receptors. In addition, ATP triggers calcium influx in PC12 cells through another P_2-receptor. Activation of P_{2y}- but not P_{2x}-receptors leads to a sustained elevation of intracellular calcium presumably via CRAC channele, an activation of calcium-dependent potassium channels and release of norepinephrine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31104-26 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enzymatic Oxidation of Drugs to Toxic and Carcinogenic Metabolites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. M. Jerina	Section Chief	LBC, NIDDK
Others:	J. M. Sayer	Research Chemist	LBC, NIDDK
	H. Yagi	Visiting Scientist	LBC, NIDDK
	A. M. Cheh	Research Chemist	LBC, NIDDK
	N. T. Nashed	Research Chemist	LBC, NIDDK
	M. K. Lakshman	Visiting Fellow	LBC, NIDDK
	T. V. S. Rao	IRTA Fellow	LBC, NIDDK
	W. Xiao	Special Volunteer	LBC, NIDDK

COOPERATING UNITS *(if any)*

A. Conney, Rutgers U. (Newark, NJ); W. Levin, Roche Res. Center (Nutley, NJ); D. Boyd, Queen's U. Belfast (N. Ireland); A. Dipple, FCRDC (Frederick, MD); D. Whalen, U. MD (Baltimore); H. Yeh, LAC, NIDDK; D. Gorenstein, Purdue U. (W. Lafayette, IN)

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Oxidation Mechanisms

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8	7	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary goal of this research project has been the elucidation of the structures and mechanisms of action of reactive metabolites responsible for the carcinogenic, cytotoxic and mutagenic activity of drugs, polycyclic aromatic hydrocarbons and other environmental chemicals. Intensive studies over a several year period have resulted in the identification of bay-region diol epoxides as the metabolites responsible for the tumorigenic activity of the polycyclic aromatic hydrocarbons. Synthetic studies during the past year have produced biologically significant oligonucleotides containing specific diol epoxide adducts. Modified oligonucleotides corresponding to trans opening of the (+)-(7R,8S)-diol (9S,10R)-epoxide of benzo[a]pyrene (BPDE) and its (-)-enantiomer by the exocyclic amino group of the central deoxyadenosine (dA) in codons 60-62 of the human K-ras b oncogene were synthesized. Duplexes formed with the complementary 9-mer have melting points of 16 and 28 degrees C for the adducts of the (+)- and (-)-BPDE, respectively. However, when the central thymidine (T) of the complementary strand was replaced by deoxyguanosine (dG), both duplexes melted at 28 degrees. The more thermodynamically stable dG mismatch was thus a more suitable structure for conformational studies by NMR spectroscopy. Our NMR data indicate that the hydrocarbon portion of both diol epoxides is intercalated into the DNA helix. In addition, the adducted dA from the (+)-enantiomer adopts an unusual syn-conformation and is hydrogen bonded to the dG opposite it. This contrasts with earlier NMR studies of dG adducts of these same diol epoxides in which the hydrocarbon was found to lie either up or down in the minor groove of the DNA. Both enantiomeric diol epoxides are potent mutagens in bacterial and mammalian test systems. Although mutations at dG predominate from both enantiomers at high doses of BPDE, at very much lower and environmentally relevant doses, the much more carcinogenic (+)-enantiomer shows a marked increase in base substitutions at AT compared to GC base pairs. This increase is not seen with the (-)-enantiomer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK31106-07 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanistic Enzymology of HIV Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	D. M. Jerina	Section Chief	LBC, NIDDK
Others:	J. M. Sayer	Research Chemist	LBC, NIDDK
	N. T. Nashed	Research Chemist	LBC, NIDDK
	M. T. Haber	IRTA Fellow	LBC, NIDDK
	T. V. S. Rao	IRTA Fellow	LBC, NIDDK

COOPERATING UNITS *(if any)*

J. M. Louis, E. Wondrak and A. R. Kimmel, LCDB, NIDDK; K. D. Parris, LMB, NIDDK

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Oxidation Mechanisms

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.1	3.0	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mechanisms of action of the reverse transcriptase (RT) and protease of human immunodeficiency virus type 1 (HIV-1) are being investigated with the goal of developing specific inhibitors for these enzymes. i) Autoprocessing of the HIV-1 protease from a construct, MBP-TF-PR-Pol, containing the protease sequence (PR) flanked by a 19-amino acid Pol sequence at the C-terminus and a 12-amino acid sequence from the trans frame peptide (TF) at the N-terminus, fused to the maltose binding protein (MBP) of E. coli, involves two sequential proteolytic steps: cleavage at the N-terminus of the protease sequence to release a 13.2-kDa protein intermediate followed by C-terminal cleavage to give the mature, 11-kDa protease. The purified 13.2-kDa intermediate is virtually identical to the mature protease in its kinetic behavior, but it is somewhat more sensitive to denaturation by urea than the mature enzyme. There is no evidence for dissociation of the enzymatically active, dimeric form of this intermediate to inactive monomers at protein concentrations above 25 nM. Since the spontaneous conversion of the intermediate to the mature protease is second-order in protein concentation, its mechanism probably involves hydrolysis of the C-terminal fragment of one dimeric protein by a second dimer. ii) Kinetics of steady-state incorporation of a single nucleotide residue derived from thymidine or 4-thiothymidine triphosphate (TTP or 4S-TTP respectively) into a RNA-DNA template-primer by RT from HIV-1 were compared with those for the Klenow fragment of E. coli DNA polymerase I with a DNA template-primer. With the Klenow fragment, the Michaelis constant for 4S-TTP is 3.5 times larger than that for TTP, whereas with HIV-1 RT, the Michaelis constants for the two triphosphates are essentially identical. With the Klenow fragment, the rate constant for turnover of the enzyme is ca. 5 times larger with 4S-TTP than with its oxygen-containing analog, whereas with RT this rate constant for 4S-TTP is half that observed for TTP. The differences in turnover presumably reflect differences in rates of dissociation of the oxygen- and sulfur-containing oligonucleotide products from the enzymes, since this step is likely to be rate-determining for the enzymatic reaction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31108-06 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Adenosine Receptor Agonists and Antagonists

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and

P.I.	J.W. Daly	Chief	LBC, NIDDK
Others:	K. Hutchinson	IRTA	LBC, NIDDK
	Y. Shin	IRTA	LBC, NIDDK
	W. Padgett	Biologist	LBC, NIDDK
	Dan Shi	Special Volunteer	LBC, NIDDK

COOPERATING UNITS (if any)

R. Olsson, U. So. Fla., Tampa, FL; C. Muller, U. Tubingen, Germany; B. Fredholm, Karolinska Inst., Stockholm, Sweden.

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Pharmacodynamics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.1	2.8	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
   ☐ (a1) Minors
   ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Adenosine regulates a wide range of physiological functions through interaction with at least two major classes of adenosine receptors. The A1 and A3 classes of adenosine receptors are inhibitory to adenylate cyclase, while the A2 class is stimulatory to adenylate cyclase. Subclasses of adenosine receptors also occur. Some of these are inhibitory to calcium channels, some are stimulatory to potassium channels, some activate guanylate cyclase, some modulate phospholipid turn-over and some cause smooth muscle relaxation. A series of pyrazolopyridines were synthesized. The ethyl 1-ethyl-4-butylaminopyrazolopyridine-5-carboxylate was a non-specific A_1- and A_{2a}-adenosine receptor antagonist with a potency some 50-fold greater than theophylline. Adenoregulin, a peptide isolated from an Amazonian hylid frog and mastoparan, a wasp peptide, both markedly stimulate binding of agonists to A_1, α_2 and 5-HT_{1A} receptors. All of these receptors are coupled via G_i-class proteins to adenylate cyclase. Both adenoregulin and mastoparan enhance turnover of guanylnucleotides at Gi proteins. It appears that enhancement of binding of agonists to receptors by these peptides is due to conversion of the receptor-G-protein complex to a high-affinity guanyl nucleotide-free state. No evidence for direct functional activation of phospholipase C by adenoregulin or mastoparan was obtained. Instead, the peptides were inhibitory to phosphoinositide breakdown in permeabilized cells. Chronic caffeine ingestion by mice causes increases in density of A_1-adenosine, serotonin, cholinergic and $GABA_A$-receptors and L-type calcium channels and decreases in β-adrenergic receptors. A_{2a}-Adenosine, α-adrenergic, dopaminergic and NMDA receptors appear unaltered. Behavioral responses to adenosine analogs, caffeine, cholinergic and dopaminergic agents are altered after chronic caffeine. The behavioral depressant effects of ethanol are markedly decreased after chronic caffeine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 31110-18 LBC

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Analogues of Thyrotropin-releasing Hormone

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Louis A. Cohen	Chief, Section on Biochem. Mechanisms	NIDDK-LBC

COOPERATING UNITS (if any)
Dr. H. Kimoto, Nagoya, Japan; v. Labroo, Seattle, WA; S. Vonhof, USUHS; A.Faden, Washington, D. C.

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is temporarily inactive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31111-24 LBC

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Stereopopulation Control in Drug Delivery and Enzyme Simulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIDDK-LBC
Other:	Michael M. King	Special Volunteer	GWU

COOPERATING UNITS *(if any)* Y. Ueno, Nagoya, Japan; Y. Takeuchi, Toyama, Japan; W. Antkowiak, Poznan, Poland; J. Flippen-Anderson, NRL, Washington, DC

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have synthesized a wide variety of intramolecular systems which simulate the enzyme-substrate complex by having the molecule frozen into a single conformation very favorable to bringing the interacting groups into the closest possible juxtaposition (stereopopulation control). These compounds undergo intramolecular reactions at rates approaching those catalyzed by enzymes (but independently of any functional group assistance). As part of our studies of practical applications of stereopopulation control, we have explored the use of various SPC-derivatives of biogenic amines and antibiotics as prodrugs. The intent is to facilitate transport from the gut to the circulatory system to the desired site of action by temporary masking of charge and polarity within the drug, by improvement in lipophilicity and by protection against enzymatic destruction. Release of the drug is based on local pH variation in ligand sites or on local concentration of a potent reducing enzyme. Recent studies have concentrated on o-nitrophenylpropionic acid derivatives as carriers. To date, these carriers have been coupled with GABA, DOPA derivatives and indolealkylamines. The nitro group is reduced enzymatically, and the resulting amine attacks an amide bond intramolecularly to release the drug. Rapid attack by the amine is ensured by placing a gem-dimethyl group on the adjacent carbon atom. Kinetic studies have shown that chemical reduction of the nitro group occurs at the same rate with or without the gem-dimethyl group, but that drug release is greatly enhanced due to the conformational restriction imposed by the gem-dimethyl group. Surprisingly, xanthine oxidase and various NADH-coupled enzymes reduce the hindered nitro compound more readily than the unhindered. This result reveals that the enzyme must operate on the face of the nitro group, and not just an edge. Response to other reducing agents is now being explored with a view to using such prodrugs to deliver mustards and other anticancer agents to hypoxic cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 31112-18 LBC

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Chemistry of Imidazoles and Biomidazoles

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIDDK-LBC

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

No activity during this period.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31113-18

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Halogenated Biogenic Amines in Biochemistry and Pharmacology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. Kenneth L. Kirk Chief, Section on Drug-Receptor Interactions LBC, NIDDK
Other:
Bang-Hua Chen Visiting Fellow LBC, NIDDK
Jun-ying Nie Visiting Assoc. LBC, NIDDK
Mona Singh IRTA Fellow

COOPERATING UNITS (if any)

M. Channing, D. Kiesewetter (CC, Dept. of Nuclear Medicine), D.S. Goldstein (HE, NHLBI), V. Pike (Hammersmith Hospital, London)

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Drug-Receptor Interactions

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.2	4.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Biogenic amines play key roles in neurotransmission, metabolism, and in control of various physiological processes. Using a variety of synthetic methodologies, including novel procedures developed by us, we have prepared a series of biogenic amines with fluorine substituted at various ring-positions. By virtue of its very small size and high electronegativity, fluorine is a very favorable replacement for hydrogen in these analogs. The biological properties and usefulness of these ring-fluorinated biogenic amines have proved to be extremely rewarding and continue to find applications in a multitude of studies, including research on the mechanisms of transport, storage, release, metabolism, and modes of action of these amines. Of particular significance was the discovery that 6-fluoronorepinephrine is a selective α-adrenergic agonist and 2-fluoronorepinephrine is a selective β-adrenergic agonist. Mechanisms considered to explain these results include: 1) a direct effect of the C-F bond on agonist-receptor interaction or 2) an indirect effect of the C-F bond on the conformation of the ethanolamine side-chain. The results of testing of new analogs synthesized to probe these mechanisms have not clearly differentiated between these two basic mechanisms. Binding of fluorinated analogs to cloned wild-type and mutant adrenergic receptors is now being studied to try to identify specific sites on the receptor protein responsible for fluorine-induced adrenergic selectivities. Fluorinated analogs are useful mechanistic probes and biological tracers. [18F]-labeled 6-fluorodopamine, the biological precursor to 6-fluoronorepinephrine, has been found to be an excellent scanning agent for peripheral noradrenergic innervation. We have developed stereoselective syntheses of threo-2- and 6-fluorodihydroxyphenylserine (fluoro-DOPS). These will be studied as prodrugs for the elaboration of 2-and 6-fluoronorepinephrine in the central nervous system. Diasteroselctive and enantioselective approaches to [18F]-labelled-threo-6-fluoro-DOPS as a PET-scanning agent for central adrenergic innervation have been developed. A new synthesis of fluorinated and polyfluorinated veratraldehydes, based on direct electrophilic fluorination, has been realized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 31114-12 LBC

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Significance of Ligand Tautomerism in Biorecognition

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIDDK-LBC

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

No activity during this period

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31115-11 LBC

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Funtionalized Congeners of Bioactive Compounds

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. A. Jacobson	Research Chemist	LBC, NIDDK
Others:	Jeong, Hea OK	Visiting Fellow	LBC, NIDDK
	Ji, Xiao Duo	Visiting Associate	LBC, NIDDK
	Kim, Jeongho	Visiting Fellow	LBC, NIDDK
	Melman, Neli	Special Volunteer	LBC, NIDDK
	Sanders, Lawrence	Summer IRTA	LBC, NIDDK
	Siddiqi, Suhaib M.	IRTA Fellow	LBC, NIDDK
	Van Rhee, Albert M.	Special Volunteer	LBC, NIDDK

COOPERATING UNITS (if any) A. Ijzerman (Univ. Leiden, Netherlands); G. Stiles (Duke Univ.); Y. Karton (Israel Inst. Biol. Res, Nes Ziona, Israel); K. Harden (Univ. N. Carolina); G. Burnstock (U. College London)

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Molecular Recognition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.6	4.3	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recent work in our laboratory has demonstrated that certain drugs may be attached to well-defined "carrier" molecules and still retain the ability to bind to the receptor site and effect biological activity. This synthetic strategy for the attachment of drugs to carriers is termed the "functionalized congener" approach. The "carrier" molecule may be many times larger than the parent drug; indeed there is practically no maximum size limitation for a fully potent analog. Unlike the prodrug approach or the immobilization of drugs for slow release, the "functionalized congener" approach is designed to produce analogs for which no metabolic cleavage step is necessary for activation. Moreover, the attachment of the drug to a "carrier" such as a peptide may result in enhanced affinity at an extracellular receptor site and an improvement in the pharmacological profile of the parent drug.

Purine derivatives containing attached chains were developed as functionalized congeners for adenosine receptors and for ATP receptors. Reporter groups such as fluorescent dyes have been covalently attached resulting in receptor probes of relatively high affinity. Sites for chain derivatization on the structures of ATP and selective muscarinic antagonists have been located. A3 adenosine receptors are important in the regulation of CNS, cardiac, inflammatory, and reproductive functions. We have developed the only selective agents for this novel receptor. Selective A3 agonists are effective in the treatment of neurodegenerative diseases. Antagonists have been proposed to have anti-inflammatory properties. New xanthine and adenosine derivatives are being synthesized, screened for A3-receptor selectivity, and later tested in vivo. Irreversibly-binding receptor probes are being developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31116-07 LBC

PERIOD COVERED

October 1, 1993 to september 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Prosthetic Groups for Labeling of Functionalized Drugs and Peptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: K. A. Jacobson	Research Chemist	LBC, NIDDK

COOPERATING UNITS (if any)

P. Skolnick (NIDDK); J. Baumgold (NIDDK); M. Channing (NM-CC); G. Stiles (Duke University)

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Molecular Recognition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The use of radioisotopes to label organic compounds for use in diagnostic nuclear medicine is well documented in the literature. It has been found that certain radiolabeled compounds will localize in the brain, heart, or in other target organs or tissues to a sufficient level to allow for imaging thereof.

Binding sites for certain drugs in an animal or organ may be localized as a result of the synthesis of high specific activity radiolabeled analogs which have high affinity for that binding site. Prosthetic groups may be attached to a drug or other receptor ligand for the purpose of efficient and selective chemical capture of a particular radioisotope. Having developed functionalized congeners of theophylline and other drugs acting at adenosine receptors, we are now developing prosthetic groups for radioisotopes such as 18-F, 123-I, and 125-I, to be coupled to these functionalized drug molecules. The prosthetic groups contain amino or carboxylic groups which are to be condensed covalently to functionalized drugs to give conjugates of high affinity at a particular receptor, or drugs that bind the label irreversibly (trifunctional reagents).

A fluorescein conjugate, FITC-APEC, was derived from a series of functionalized congeners that act as selective A2a-adenosine agonists. The binding of FITC-APEC to brain striatal A2a-adenosine receptors measured by fluorescence techniques was saturable and of high affinity. The potencies of chemically diverse A2-adenosine receptor ligands estimated by inhibition of FITC-APEC binding were in good agreement with their potencies determined using radioligand binding techniques. These findings validate the use of this novel fluorescent ligand to quantitatively characterize binding to A2-adenosine receptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31117-07 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Drugs Acting at Adenosine Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. A. Jacobson	Research Chemist	LBC, NIDDK
Others:	M. Beenhakker	Special Volunteer	LBC, NIDDK
	M. Carter	Marc Student	LBC, NIDDK
	Xiao Duo Ji	Visiting Associate	LBC, NIDDK
	Jeongho Kim	Visiting Fellow	LBC, NIDDK
	Neli Melman	Special Volunteer	LBC, NIDDK
	Dag Von Lubitz	Guest Researcher	LBC, NIDDK

COOPERATING UNITS (if any) A. Shainberg (Bar Ilan Univ., Israel); G. Stiles (Duke Univ.); L. Belardinelli (U. Florida); R.A. Olsson (Univ. So. Florida); K. LaNoue (Univ. Penn); A.P. Ijzerman (Ctr. for Bio-PHarmaceut. Sci., Leiden, The Netherlands)

LAB/BRANCH

Laboratory of Bioorganic chemistry

SECTION

Molecular Recognition

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.8	2.1	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The extracellular adenosine receptor has a modulatory role in the nervous, circulatory, endocrine, and immunological systems. The prospect of harnessing these effects specifically for therapeutic purposes is attractive. Recently this project has focused on the effects of adenosine agonists and antagonists in the central nervous system and on the possibility of therapeutics for treating neurodegenerative diseases. Chronic treatment with an adenosine agonist improves spaital memory retention and acquisition and indicates necessity of further studies directed towards Alzheimer's disease. Neuroprotective effects of acutely administered A1 receptor agonists and chronically administered A1 receptor antagonists have been demonstrated. Adenosine agonists prevent convulsions in several chemical and electrical seizure models and protect against excitotoxic neurodegeneration agonists and antagonists suggests some adaptation (sensitization by antagonists/desensitization by agonists) of the mechanism of receptor activation, either at the level of receptor or second messenger. An A3 agonist proved to be highly cerebroprotective in an ischemic model in gerbils. In summary, highly selective adenosine analogues may have therapeutic potential in treatment of cerebral ischemia/stroke and possibly other neurodegenerative disorders as well. Since the three major subtypes of adenosine receptors have been cloned it has been possible to conduct molecular modeling of the receptor protein, based on sequence analyses and computerized energy minimizations. A hypothesis concerning the mode of binding to ligands to adenosine receptors has been derived. This hypothesis is consistent with pharmacological observations and site directed mutagenesis experiments, in which key histidyl residues have been replaced by other amino acids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31118-05 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bioindoles and Oxindoles as Medicinal and Diagnostic Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIDDK-LBC
Other:	Yigal Fraenkel	IRTA Fellow (term 11/93)	NIDDK-LBC
	R. G. Rajeswaran	Visiting Fellow (EOD 6/94)	NIDDK-LBC
	Karen Shafer	Summer IRTA	NIDDK-LBC

COOPERATING UNITS *(if any)* Peter Kador, NEI; J. Flippen-Anderson, NRL, Washington, DC; R. Labroo, Seattle, WA; P. A. Cohen, Vancouver, B.C.; Sanford Markey, NIMH; David Klein, NICHD; Robert Phillips, Athens, GA; S. Gronowitz, Lund, Sweden

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.8	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Interest in the physiological activities and potential clinical uses of the pineal hormone melatonin (N-acetyl-5-methoxytryptamine) has grown enormously in recent years. The hormone has been implicated in the regulation of phenomena as diverse as the sleep-wake cycle, depression, sexual urge, neuroimmunomodulation, mammary cancer, etc. Such multiplicity of actions in different tissues suggests a multiplicity of receptors for the same molecule. We plan to test this possibility by photoaffinity labeling with azido analogs of melatonin in which the azido function is found in different positions in the molecule. Identification of the protein sequence carrying the ligand will then determine if the receptor proteins are identical or not. To this end, the N-acetyl group of melatonin has been replaced by the azidoacetyl group. Efforts will now be made to radioiodinate the new analogue.

We have found that the universally used "iodogen" method for radioiodination suffers from very low yields and often leads to competitive chlorination. We have developed an alternative method in which hydrogen peroxide is used to oxidize iodide ion in a two-phase system. This method not only avoids the expensive iodogen and the danger of chlorination, but also provides considerably improved yields of iodinated material.

Previous efforts to produce 2-fluoro analogues of indoles, by exchange of other halogens or by direct fluorination, were minimally successful. We now have found that commercial morpholinosulfurtrifluoride will replace the carbonyl group of an oxindole with fluorine, providing the first and highly practical route to 2-fluoro derivatives of bioindoles. These compounds are expected to serve as irreversible affinity labels at brain receptors. Structure-activity studies show that bulky, lipophilic substituents at C-2 improve the binding of melatonin. Methods which were highly successful in producing 2-alkylhistidines and histamines are now being applied to produce 2-alkyl derivatives of bioindoles.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31119-05 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Novel Amino Acids for Conformational and Stereochemical Constraints in Peptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIDDK-LBC

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

No activity this year

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31120-05 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Fluorinated Analogues of Bioactive Peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIDDK-LBC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been temporarily discontinued

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31121-04 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT [illegible]

Chemistry and Biology of Novel Pyrimidine and Purine Nucleosides

PRINCIPAL INVESTIGATOR [illegible]

P.I.:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIDDK-LBC

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

No activity during this period.

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 31122-04 LBC

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Medicinal Agents Based on Bioheteroaromatic Ring Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIDDK-LBC
Other:	Rahul Jain	Visiting Fellow	NIDDK-LBC
	Tuan Tran	Stay-in-school	NIDDK-LBC
	Michael King	Special Volunteer	GWU

COOPERATING UNITS *(if any)*
J. Golenser, Jerusalem, Israel; Dept. of Parasitology, WRAIR, Washington, DC

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.3	0.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have found 2-iodo-L-histidine to be a potent antimalarial agent against drug-resistant Plasmodium falciparum. The compound is effective in monkeys for only 24-48 hrs, and we have shown that inactivation may be the result of nonenzymatic deiodination by any sulfhydryl compound present in serum or tissue. The facts that 2-iodohistidine does not block protein synthesis in the parasite, and that the corresponding 2-bromo and 2-chloro compounds are inactive, led us to speculate that the 2-iodo compound operates by plugging one or more holes in the erythrocyte membrane and, thus, depriving the parasite of nonamino acid nutrients obtained by diffusion through such holes. We have, therefore, synthesized and tested a broad range of more metabolically stable derivatives of histidine and histamine, in which the ring substituents are close to iodine in size. Alkylation at C-2 is achieved by generation of an alkyl radical by one of several methods. In acidic media, the reaction is remarkably specific for C-2, providing the most efficient and least expensive method yet developed for synthesis of 2-alkylhistidines and histamines. In vitro activity appears to depend on the extent to which a substituent projects from the imidazole ring, with the optimum size being somewhat larger than that of iodine. On this basis, covalent affinity labels have been designed and are being prepared. Drug resistance by the parasite and multidrug resistance in cancer chemotherapy both involve drug ejection by an ATP-driven protein pump; in both cases, resistance can be partially overcome by calcium channel blockers. Accordingly, a number of our antimalarial candidates have been submitted for testing to overcome multidrug resistance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 32001-3LBC

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Development of Multifuntional Chemotherapeutic Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. Vithal Shetty	Guest Researcher	LBC, NIDDK
Others:			
	K. L. Kirk	Chief, Section on Drug-Receptor Interactions	LBC, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Drug-Receptor Interactions

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A novel approach to the design of new antimicrobial agents has been developed. The overall purpose of the program is the rational design and synthesis of compounds that have combined anti-bacterial, anti-viral and anti-fungal activity. Specifically targeted in the rational design are enveloped viruses such as herpes viruses and HIV. These compounds have novel structural features which include a dimeric attachment of a known hydrophobic antimetabolite (for example, aminoadamantane analogues) through a very hydrophilic bridge. These structural features combine to provide the potential for a novel mechanism for attachment of the toxic moiety to microbial organisms. Thus, the hydrophilic portion of the molecule has a high affinity for glycoprotein components of the microbial cell wall. This affinity will deliver the toxic moieties to the cell surface, providing a mechanism for efficient activity. For example, the potential exists for inhibition of replication of such viruses as HIV and, through the attachment of the toxic molecule to the viral coat, a mechanism for killing the virus.

Initial testing of several bisadamantamine analogues (U.S. Patent No. 5,221,693) revealed potent activity against gram positive and gram negative bacteria, fungi, yeast and enveloped viruses. Such combined activity potentially could be extremely useful in treatment of immunosuppressed patients. Current modifications to these prototypical molecules include introduction of a sulfoxide group in the bridging alkylene group to provide increased hydrophilic interactions. Also, aromatic rings have been introduced adjacent to the adamantane rings. These modifications have led to marked improvement in the level of anti-viral activity, and concomitant reduce cytotoxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 32002-02 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synthesis and Biochemistry of Ascorbic Acid Analogues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kenneth L. Kirk	Chief, Section on Drug Receptor Interactions	LBC, NIDDK
Others:	Arthur T. Crossman, Jr.	IRTA FELLOW	LBC, NIDDK

COOPERATING UNITS *(if any)*

Mark Levine, Richard Welch, Yaohui Wang (Nutrition Program, LCBG, NIDDK)

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Drug-Receptor Interactions

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ascorbic acid (vitamin C), a dietary requirement for human health, is an electron donor for several enzymatic actions, functions as an antioxidant, and is implicated in host defense mechanisms, endocrine function and the visual process (lens). Recent renewed interest in the biochemistry of ascorbic acid has been prompted by the realization that relatively little is known concerning the concentrations of the vitamin required for optimum functioning of these several roles. In the case of enzymatic reactions, optimal rate of a process is defined as that concentration that allows the reaction to reach Vmax without toxicity. As part of a program to determine these concentrations, in situ kinetic measurements have been carried out for certain vitamin C-linked reactions. In addition to examination of functional roles of vitamin C, recent characterization of efficient transport mechanisms that translocate vitamin C across cellular membranes has emphasized the importance of the vitamin to biological processes. Kinetic parameters of these transport mechanisms are also being determined.

As part of a program to study the effects of structural modification on vitamin C function and to provide biochemical tools for vitamin C research, we have prepared the known 6-halo-6-deoxy analogues. Kinetic measurements of transport inhibition of these analogues have clearly demonstrated the presence of separate pathways for the translocation of ascorbic acid and dehydroascorbic acid.

We have now developed routes to 6-aryloxy-ascorbic acid analogues through displacement of triflate esters of gulonolactone intermediates. This versatile procedure has premitted introduction of functional groups on the 6-aryl ring. Using such functionalized analogues as strarting points, various tracers and affinity labels will be introduced.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 32003-02 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Neurotransmitter Receptor Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Jürgen Wess, Ph.D.	Visiting Scientist	LBC, NIDDK
Others:	Klaus Blüml	Special Volunteer	LBC, NIDDK
	Nathalie Blin, Ph.D.	Visiting Fellow	LBC, NIDDK
	Jie Liu, Ph.D.	IRTA Fellow	LBC, NIDDK

COOPERATING UNITS (if any)

C. Felder, Ph.D., LCB, NIMH
B. Conklin, Ph.D., University of California, San Francisco

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Drug Receptor Interactions

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The five muscarinic acetylcholine receptors (m1-m5) are members of a superfamily of plasma membrane receptors which regulate cellular activity via coupling to heterotrimeric G proteins. We have used the muscarinic receptors as model systems to study how G protein-coupled receptors function at a molecular level. The molecular mechanisms involved in receptor assembly, ligand binding, and G protein coupling were studied by using a combined pharmacologic/molecular genetic approach.

Functional analysis of a series of hybrid m2/m5 muscarinic receptors showed that the three-dimensional structure of muscarinic receptors (and, most likely, other G protein-coupled receptors) is similar to that of bacteriorhodopsin and that direct molecular interactions between TM I and TM VII are required for proper receptor folding.

Mutational analysis of the m3 muscarinic receptor demonstrated that a TM VI Asn residue (Asn507) which is characteristic for the muscarinic receptor family plays a specific role in the binding of certain subclasses of muscarinic antagonists including atropine-like agents and pirenzepine. Replacement of Asn507 by Ala, Ser, or Asp led to mutant m3 receptors with drastically reduced binding affinities (up to 28,000-fold) for these ligands.

Previous studies have shown that the N-terminal segment of the third intracellular loop (i3) is critically involved in determining the G protein coupling properties of the individual muscarinic receptor subtypes. Using a novel insertion mutagenesis approach, we could demonstrate that this region forms an amphiphilic α-helical extension of TM V and that one side of this helix (most likely the hydrophobic one) represents a major G protein recognition surface. Systematic mutational modification of the N-terminal segment of the i3 loop of the rat m3 muscarinic receptor showed that a tyrosine residue (Tyr254) located at the beginning of this domain is essential for efficient activation of Gq (mediated by the m1, m3, and m5 muscarinic receptors).

These studies should eventually lead to a detailed structural model of the ligand-receptor-G protein complex which should provide a rational basis for the development of novel muscarinic drugs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 33000 28 LMB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies of Functions Involved in Genetic Recombination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: Martin Gellert, Ph.D., Chief, Section on Molecular Genetics, LMB/NIDDK

OTHERS: Sergei Kozyavkin, Ph.D., Visiting Associate, LMB/NIDDK
Regis Krah, Ph.D., IRTA Fellow, LMB/NIDDK
Mary H. O'Dea, Research Chemist, LMB/NIDDK

COOPERATING UNITS (if any)
Dr. Rolf Menzel, Bristol-Myers Squibb, Princeton, NJ

LAB/BRANCH
Laboratory of Molecular Biology, DIR/NIDDK

SECTION
Section on Molecular Genetics

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.6	2.6	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Reverse DNA gyrases are enzymes found in thermophilic bacteria, whose action introduces positive supercoiling into circular DNA at the expense of ATP hydrolysis. Previously described enzymes of this class have a single polypeptide chain. We have now found a reverse gyrase with an unusual structure in the hyperthermophile Methanopyrus kandleri. This enzyme has two subunits, one having the ATPase activity while the other carries out the DNA breakage-reunion step. This functional partition is similar to that of the eubacterial DNA gyrases.

Further studies of E. coli DNA gyrase have shown that the coupling between supercoiling and nucleotide triphosphate consumption depends on the initial supercoiling of the DNA. Tight coupling can be obtained if the DNA is initially positively supercoiled.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 33001 10 LMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Immunoglobulin Gene Rearrangement

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: Martin Gellert, Ph.D., Chief, Section on Molecular Genetics, LMB/NIDDK

OTHERS: Joanne Hesse, Ph.D., Research Chemist, LMB/NIDDK
Fraser McBlane, Ph.D., Visiting Fellow, LMB/NIDDK
Moshe Sadofsky, M.D., Ph.D. Sr. Staff Fellow, LMB/NIDDK
Dik Van Gent, Ph.D., Special Volunteer, LMB/NIDDK
Dale Ramsden, Ph.D., Special Volunteer, LMB/NIDDK

COOPERATING UNITS (If any)

Dr. M.J. Bosma, Fox Chase Cancer Center

LAB/BRANCH

Laboratory of Molecular Biology, DIR/NIDDK

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.8	5.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The products of the RAG-1 and RAG-2 genes are known to be the only lymphoid-specific factors required for the rearrangement of antigen receptor genes (generally known as V(D)J recombination). We have made a large number of mutations in the mouse RAG-1 and RAG-2 genes, for the purpose of understanding the functions of different parts of the proteins. So far, we have learned that large parts of both proteins are dispensable for recombination activity. The N-terminal 35% of RAG-1 can be removed without a significant decrease of activity; similarly, the C-terminal 25% of RAG-2 is not essential. The deleted regions include a zinc-finger motif in RAG-1 and a highly acidic sequence in RAG-2, which are thus shown to be non-essential.

One mutation in RAG-1 has been shown to allow recombination of some substrates but not others, providing the first positive evidence that RAG-1 interacts directly with the recombination sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 33006-16 LMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Mechanism of Genetic Recombination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: Kiyoshi Mizuuchi, Ph.D., Chief, Section on Genetic Mechanisms, LMB/NIDDK

OTHERS: M. Mizuuchi, Ph.D., Visiting Associate, LMB/NIDDK
H. Savilahti, Ph.D., Visiting Fellow, LMB/NIDDK
P. Rice, Ph.D., IRTA Fellow, LMB/NIDDK

COOPERATING UNITS (if any)

M. Clore, Ph.D., LCP/NIDDK, A. Gronenborn, Ph.D., LCP/NIDDK
J. Omichinski, Ph.D., LCP/NIDDK, R. Clubb, Ph.D., LCP/NIDDK

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Genetic Mechanisms

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major objective of this project is to uncover the molecular mechanisms of a variety of genetic rearrangements. The transposition reaction of bacteriophage Mu is studied as a model system.

Critical steps in Mu transposition are a pair of DNA cleavages and strand transfers which generate a branched DNA intermediate. Efficient formation of this intermediate requires, besides the MuA protein which is the transposase, the following accessory factors: the MuB protein, the *E. coli*-encoded HU and IHF proteins, ATP, and Mg^{++}. MuA interacts with two different specific DNA sequences, one at the ends of the Mu genome and the other at the Mu operator. Interactions involving multiple MuA molecules, accessory protein factors and sequence elements on the donor DNA lead to formation of a stable protein-DNA complex in which the two Mu ends are synapsed by a tetramer of MuA. Next, a pair of single strand cuts are made to expose the 3′ ends of the Mu DNA. The cleaved donor DNA remains tightly associated with the MuA tetramer and this complex efficiently captures a "target" DNA molecule provided it is bound by MuB. A staggered cut is introduced into the target DNA and the two 5′ ends are joined to the 3′ ends of the Mu DNA in a concerted reaction.

The assembly process and the functional organization of the MuA tetramer-Mu DNA complex have been studied by making use of a variety of mutant MuA proteins with missing functional domains. Structurally and functionally important protein-DNA interactions within the stable complexes were analyzed by assembling the complexes from short Mu end DNA fragments and MuA under permissive reaction conditions, bypassing the need for many of the cofactors normally required for the process. Efforts are also under way to determine the structure of MuA by combining X-ray crystallography and NMR methods.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 34002-29 LMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Enzyme Structure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: David R. Davies, Chief, Section on Molecular Structure, LMB/NIDDK

OTHERS: Mi Li, Ph.D., Visiting Associate, LMB/NIDDK
Der-Ing Liao, Ph.D., Visiting Associate, LMB/NIDDK
Kevin Parris, Ph.D., Staff Fellow, LMB/NIDDK
Enid Silverton, Ph.D., Research Chemist, LMB/NIDDK

COOPERATING UNITS (if any)

Edith Miles, Ph.D., NIDDK Alan Peterkovsky, Ph.D., NIDDK
Ira Paston, Ph.D., NCI

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Section on Molecular Structure

INSTITUTE AND LOCATION

NIDDK/NIH - Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.8	PROFESSIONAL: 2.8	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have continued work on the high resolution structure of the bifunctional enzyme complex tryptophas synthase. We have examined mutants in order to understand the mechanism of action of both enzymes and the mechanism of communication between the two active sites. The native enzyme structure has been refined to 1.9A resolution. A sodium binding site has been identified that plays a role in the activity of the enzyme.

Domain III of pseudomonas Aeruginosa exotoxin has been crystallized and the structure determined and the refinement is now almost complete. The enzyme was crystallized with NAD and the location of the NAD binding site has been determined, as part of the goal of understanding the mechanism of action.

The 30kD N-terminal domain of enzyme 1 of (PTS), the phosphoenolpyruvate: sugarphosphotransferase system has been crystallized and the determination of the crystal is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 34003 26 LMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Three-dimensional Structures of Cytokines, Receptors and Immune System Proteins.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: David R. Davies, Ph.D., Chief, Section onMolecular Structure, NIDDK/LMB

Others: Peter Sun, Ph.D., Visiting Scientist, NIDDK/LMB - Susan Chacko, Ph.D., IRTA, NIDDK/LMB - Enid Silverton, Ph.D., Research Chemist, NIDDK/LMB - Gerson H. Cohen, Ph.D., Research Chemist, NIDDK/LMB - Der-Ing Liao, Ph.D., Visiting Associate, NIDDK/LMB

COOPERATING UNITS (if any)

Warren Leonard, Ph.D., NHLBI

LAB/BRANCH

Laboratory of Molecular Biology/NIDDK

SECTION

Section on Molecular Structure

INSTITUTE AND LOCATION

NIDDK/NIH - Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.7	3.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1) Small crystals have been obtained for the low affinity complex of the cytokine,human Il-2 and its receptor, IL-2Rα.

2) The crystal structure of a catalytic antibody that catalyses a porphyrin metallation reaction has been determined.

3) The TGF-β previously determined by us has been shown to belong to an extensive superfamily of 'cystine-knot' cytokines.

4) The complex of HyHEL-5 Fab with various lysozymes have been crystallized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 35000-30 LMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chemical and Structural Investigations of Nucleic Acids and Related Molecules

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	H. Todd Miles, Ph.D.	Chief, Section on Organic Chemistry	LMB/NIDDK
OTHERS:	V. Sasisekharan, Ph.D.	Visiting Scientist	LMB/NIDDK
	F.B. Howard, Ph.D.	Research Chemist	LMB/NIDDK
	J. Frazier	Research Chemist	LMB/NIDDK
	Keliang Liu, Ph.D.	Visiting Fellow	LMB/NIDDK

COOPERATING UNITS (if any)

David R. Davies, LMB/NIDDK
R. Raghunathan, NCI/NIDDK
Robert Langer, MIT
Philip Ross LMB/NIDDK
Girjesh Govil, TIFR, India

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Organic Chemistry

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	5.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have designed parallel stranded AT DNA structures constrained to parallel polarity by terminal hemiprotonated CC^+ 'clamps'. The helices have reverse Watson-Crick pairing. These constructs were used to characterize and calibrate the same helical sequences without the terminal CC^+ pairs. The data also show that CC^+ helices can be constrained to duplexes rather than the four stranded structures recently shown for unconstrained acid oligo dC.

We have obtained the first crystals of DNA triple helices and observed fiber type X-ray diffraction patterns. Molecular models were constructed and compared with Fourier transforms calculated from the diffraction patterns. Very good agreement with the experimental data was obtained for the two kinds of observed B form structures, one with 12 and one with 13 residues per turn.

The DNA restriction sequence d-GGTACGCGTACC and its 6,7 IA mispair analog form duplex helices in equilibrium with monomer hairpins. We proposed that the hairpins had two-base loops, not previously demonstrated. We have now shown by NMR spectroscopy the loop size is two rather than four and have determined other aspects of the structures of helix and duplex.

Dr. V. Sasisekharan has developed a new method of modeling polysaccharide structures. This approach has led to several stereochemically satisfactory structures of glycosaminoglycans which agree with experimental data. Previous structures had not agreed with experiment and had non-allowed short contacts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36003-10 LMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nonheritable Antibiotic Resistance

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: J. L. Rosner, Ph.D., Research Biologist, LMB/NIDDK

COOPERATING UNITS (If any)

R. G. Martin, M.D., Medical Researcher, LMB/NIDDK G. Storz, Ph.D., CBMB/NICHD

LAB/BRANCH

Laborator of Molecular Biology

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Important roles for several microbial stress response systems in antibiotic resistance have been found.

A. Independent regulation of antibiotic resistance gene by the *marRAB* (multiple antibiotic resistance) and *soxRS* (superoxide) stress response systems of *Escherichia coli* has been demonstrated. When the *marRAB* operon is transcriptionally activated due to exposure to certain antibiotics (chloramphenicol, tetracycline), aromatic weak acids (salicylate, 2,4-dinitrophenol) or mutations in the *marR* (repressor) gene, the *marA* product activates the transcription of a set of genes which helps the cells resist antibiotics and superoxides. The *soxRS* system which is activated by exposure to superoxide generating agents and nitric oxide also activates this set of genes by means of the SoxS transcriptional activator. The present work demonstrates that the two systems operate largely independently: deletion of *marRAB* does not prevent induction of *soxRS* by paraquat while deletion of *soxRS* does not prevent induction of *marRAB* by salicylate. In addition, the existence of a *marRAB*-independent pathway for induction of antibiotic resistance by salicylate has now been established.

B. *Escherichia coli* and *Salmonella typhimurium* are resistant to Ÿ1 mg/ml concentrations of isoniazid (INH), a clinically important antituberculosis drug. The present work has shown that resistance is largely due to the function of peroxidases controlled by the OxyR peroxide response system. Mutants with defective *oxyR* (encoding the transcriptional regulator) or *katG* (encoding a hydroperoxidase) and *ahp* (encoding an alkyl hydroperoxide reductase) are susceptible to as little as 50 μg/ml of INH. The role of these genes appears to be to reduce the levels of peroxides within the cell. The addition of exogenous peroxides to resistant *E. coli* or *Mycobacterium smegmatis* increases their INH susceptibility while antioxidants reduces the susceptibility of sensitive cells. These results suggest that peroxides are needed to interact with INH to generate toxicity in both *E. coli* and *M. smegmatis*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36104-13 LMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Thermal Measurements of Biomolecular Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: Philip D. Ross, Ph.D. Research Chemist LMB/NIDDK

OTHERS: Frank B. Howard Research Chemist LMB/NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biology, DIR/NIDDK

SECTION

Section on Physical Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2	PROFESSIONAL: 2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

On account of renewed interest in triple-stranded nucleic acid complexes we are carrying out a comprehensive thermodynamic study of helix formation among DNA homopolymers containing adenine, thymine and uracil. We have completed a calorimetric and spectroscopic study of the behavior of the poly(dA-poly(dT) system in the realm of high uni-univalent salt concentration where we have found the conditions for formation of a new polynucleotide complex and two entirely new helix-helix transformation reactions. The role and extent of displacement of cations, anions and water in these processes has been examined. The details of this work are being prepared for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36105-12 LMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Influences of Macromolecular Crowding on Biochemical Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	S. B. Zimmerman, Ph.D.	Research Chemist	LMB/NIDDK
OTHERS:	L. D. Murphy	Research Biologist	LMB/NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Physical Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The DNA of a bacterial chromosome is localized in a compact body called the nucleoid. Bacterial nucleoids have been extensively studied, but the basis of nucleoid stabilization has remained elusive.

The cytoplasm of _E. coli_ is very concentrated in macromolecules, giving a potential for large macromolecular crowding effects. The present results suggest that crowding-enhancement of the interaction between DNA-binding proteins and the cellular DNA may provide an important stabilizing force for the compact form of DNA in the bacterial nucleoid.

DNA-binding protein fractions from exponential and stationary phase cell extracts of _E. coli_ were isolated by affinity chromatography on native DNA-cellulose. The ability of these fractions to convert DNA into a readily-sedimented form was compared in the absence or presence of added polymers. In the absence of polymers, large amounts of the proteins were required. In the presence of polyethylene glycol or polyvinylpyrrolidone, much smaller amounts of the DNA-binding proteins were required, indicating a macromolecular crowding effect from these polymers. The enhanced binding under crowded conditions appears to resolve a paradox between the cellular abundance of the DNA-binding proteins and the amounts required in earlier in vitro studies.

The 'histone-like' protein HU from the DNA-binding protein fraction was preferentially incorporated into the pelleted DNA in the presence of polymers. Purified HU at roughly similar amounts caused a similar conversion of DNA to a readily-sedimentable ('condensed') form. Crowding-enhancement of DNA condensation by promoting the binding of proteins to the DNA provides a model for the stabilization of systems such as the bacterial nucleoid or kinetoplast DNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 36108 07 LMB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies of the Mechanism of Retroviral DNA Integration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: Robert Craigie, Ph.D., Visiting Scientist, LMB/NIDDK

Others: Alan Engelman, Ph.D., IRTA, LMB/NIDDK
Myung Soo Lee, Ph.D., IRTS, LMB/NIDDK
Tim Jenkins, Ph.D., Visiting Associate, LMB/NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Genetic Mechanisms

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Integration of a DNA copy of the viral genome into a chromosome of the host cell is a necessary step in the replication cycle of HIV and other retroviruses. The objectives of this project are to understand the detailed molecular mechanism of this step in the retroviral replication cycle. The key enzyme required for integration is the virally encoded integrase protein. Our research focusses on the biochemical activities of the HIV-1 integrase protein. Integrase is a site-specific nuclease that cleaves two nucleotides from each 3' end of the viral DNA prior to integration. Integrase also carries out the subsequent DNA strand transfer reaction that covalently inserts the viral DNA into the host genome. A major emphasis of the project is now to obtain detailed structural information on integrase. This has previously not been possible because the protein forms large heterogeneous aggregates, even at low concentration. We have made site-directed mutations in the catalytic core domain of HIV-1 integrase and identified several mutants with physical properties that make them better candidates for physical studies. One of these mutants is considerably more soluble than the wild type protein and retains catalytic activity. Structural studies of this protein are in progress. Viral DNA made in vivo by reverse transcription exists as a large nucleoprotein complex. Although integrase protein alone can carry out the enzymatic steps that insert viral DNA into host DNA in vitro, the integrity of this complex is likely to be important for the integration process in vivo. We are studying such complexes, isolated from cells infected with Moloney murine leukemia virus, to elucidate features of the integration process that have not been reproduced in simple reaction systems containing only integrase and synthetic DNA substrate. We find that the DNA within these complexes is protected against self-destructive integration into itself. This protection, which probably plays the physiological role of preventing autointegration of the viral DNA before the integration complex encounters host DNA, is conferred by a factor of cellular origin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36109 07 LMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

AIDS Related Proteins: Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: David R. Davies, Ph.D., Chief, Section on Molecular Structure, NIDDK/LMB

Others: Alison B. Hickman, Ph.D., IRTA, NIDDK/LMB
Frederick Dyda, Ph.D., IRTA, NIDDK/LMB

COOPERATING UNITS (if any)

Robert Craigie, Ph.D., NIDDK/LMB   Timothy Jenkins, Ph.D., NIDDK/LMB

LAB/BRANCH

Laboratory of Molecular Biology/NIDDK

SECTION

Section on Molecular Structure

INSTITUTE AND LOCATION

NIDDK/NIH - Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.2	PROFESSIONAL: 2.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several N- and C-terminal deletion mutants of the HIV-1 integration protein have been cloned, purified and subjected to crystallization trials. The solubility properties of two deletion forms which still retain the activity have been significantly improved with an accompanying improvement in the light scattering analysis of their oligomerization properties.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 36114-04 LMB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Structural Studies of Molecular Recognition

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.: Eduardo A. Padlan, Ph.D. Visiting Scientist LMB/NIDDK

Others: Chantal Abergel, Ph.D. Visiting Fellow LMB/NIDDK
Jennifer P. Tipper, Ph.D. Temporary GS-12 LMB/NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Molecular Structure

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(1) A molecular replacement solution of the crystal structure of the Fab of CC49, a murine monoclonal antibody against solid adenocarcinoma, has been obtained and is being refined.

(2) Small crystals of a single-chain construct containing the VL and VH of CC49 fused to human IgG1 CH2 and CH3 have been obtained.

(3) Models of the IgE-binding, lectin-homology domains of the human and murine low-affinity IgE receptors (CD23) have been built.

(4) The binding of various viral and self peptides to the murine class I MHC antigen, H-2Dd, has been modelled.

(5) A molecular replacement solution of the crystal structure of unliganded HyHEL-10 Fab has been obtained and is being refined.

(6) Crystallographic studies of several proteins have been initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36115-04 LMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of the Potential Use of Catalytic Antibodies Against AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.: Eduardo A. Padlan, Ph.D., Visiting Scientist, LMB/NIDDK

COOPERATING UNITS (If any)

Ettore Appella, M.D., Medical Officer, LCB/NCI
Birgit A. Helm, Ph.D., Lecturer, Univ. of Sheffield, UK
Thomas J. Kindt, Ph.D., Chief, LIG/NIAID

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Molecular Structure

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two peptidic transition-state analogs, corresponding to segments in the gp120 sequence, have been synthesized and used as immunogens for the production of murine monoclonal antibodies. Studies using supernatants from clones secreting antibody, incubated for varying lengths of time with transition-state analogs and native peptides, suggest that cleavage of the latter does take place. Several of those clones are being expanded. The secreted antibodies have been isotyped and are being tested for proteolytic activity against whole gp120 or gp160.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36116-03 LMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Molecular Basis of Antibiotic Resistance

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	Robert G. Martin, M.D.	Medical Officer	LMB/NIDDK
OTHERS:	Judah L. Rosner, Ph.D.	Research Biologist	LMB/NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory Molecular Biology

SECTION

Section on Microbial Genetics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Salicylates induce antibiotic resistance in E. coli via at least two pathways: one dependent upon the multiple antibiotic resistance locus, mar, and the other by a mar independent mechanism.

We had previously isolated a number of insertion mutants in a strain deleted for the mar region that failed to exhibit increased resistance to nalidixic acid in the presence of salicyl alcohol. The location of the insertion in three of these strains has now been identified. Two are independent insertions in uvrD, the gene that encodes the helicase required for repair following DNA damage. The third is located in pgm, the gene that encodes phosphoglucomutase. We have traced the failure of salicylates to induce antibiotic resistance in the pgm strains to a deficiency for glucose-1-P required for synthesis of the lipopolysaccharides of the outer membrane.

While others have reported that a 7.8 kbp mar region is necessary for the Mar phenotype, we have demonstrated that only the promoter (¢150 bp in length), the marR and the marA genes are required. Salicylate may interact directly with the marR protein since upon removal of salicylate, induction of the pathway ceases immediately. An expression vector carrying a functional MarR gene has been isolated and purification of marR is underway. An expression vector carrying a putative marA gene has also been isolated.

Goal

To identify the mechanisms of salicylate induction of antibiotic resistance in the mar and non-mar pathways.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36117-01 LMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Gene Expression in *C. elegans*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael Krause, Ph.D.	Senior Staff Fellow	LMB/NIDDK
OTHERS:	Robert Littlejohn	Biological Laboratory Technician	LMB/NIDDK

COOPERATING UNITS (if any)

Department of Embryology, Carnegie Institution of Washington (A. Fire, L. Chen)

LAB/BRANCH

Laboratory of Molecular Biology, DIR/NIDDK

SECTION

Section on Physical Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are interested in the mechanisms controlling embryonic gene expression that result in the differentiation of omnipotent cells into one of several cell fates. Our model system revolves around myogenesis during embryonic development in the nematode *C. elegans*. Specifically, we have focused on the gene encoding CeMyoD, a nematode homolog of the MyoD family of genes that were originally identified as important regulators of myogenesis in the mouse. The simplicity of the nematode, both in terms of the anatomy and the developmental complexity, allows us to study CeMyoD expression in detail. By inactivating the CeMyoD gene through mutation we have shown that, as in mammals, this gene product is required to generate functional muscle during embryogenesis. Animals that lack CeMyoD activity are paralyzed and die shortly after hatching. Because the gene encoding CeMyoD is clearly playing a critical role in determining muscle cell formation, we want to know how the expression of this gene is itself regulated during development. Our analysis of the CeMyoD gene has defined the controlling regions and we are currently attempting to find factors that act through these controlling regions. We have also started to study other regulatory factors that have been shown to be important for myogenesis in other model systems. These include factors such as the heterodimeric partner protein of the MyoD family, called E12, and an unrelated protein called Mef-2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 36118 1 LMB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Structural Analysis of Enzyme Mechanism and Specificity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: James H. Hurley, Senior Staff Fellow, LMB/NIDDK

Others: Gongyi Zhang, Visiting Fellow, LMB/NIDDK
Jay Grobler, IRTA, LMB/NIDDK

COOPERATING UNITS (if any)
Antony M. Dean, Department of Biological Chemistry, The Chicago Medical School, North Chicago, Il

LAB/BRANCH
Laboratory of Molecular Biology/NIDDK

SECTION
Section on Molecular Structure

INSTITUTE AND LOCATION
NIDDK/NIH - Bethesda, Md. 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The catalytic mechanisms and substrate specificities of decarboxylating dehydrogenases are under investigation. The structure of a closed, NAD-bound form of the decarboxylating dehydrogenase isopropylmalate dehydrogenase (IMDH) has been determined at 2.5 Å resolution by molecular replacement. This structure provides a basis for determining the origins of diverging substrate specificities among the decarboxylating dehydrogenases. Crystals have been obtained of a mutant IMDH whose substrate specificity has been altered to resemble that of isocitrate dehydrogenase (IDH). Crystals of pig heart isocitrate dehydrogenase have also been obtained.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 51,000-36 CHB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Immunology of Blood Cell Deficiencies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	N. Raphael Shulman	Chief	CHB:NIDDK
Others:	Diane M. Reid	Expert	CHB:NIDDK
	Ruyi He	Visiting Associate	CHB:NIDDK
	Charles E. Jones	Chemist	CHB:NIDDK

COOPERATING UNITS (if any)

Monica Yu (CC-DTM); Charles Carter (CC-DTM)

LAB/BRANCH Clinical Hematology Branch

SECTION

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2.0 | PROFESSIONAL: 1.5 | OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Work in the past year extended our previous findings on idiopathic thrombocytopenic purpura (ITP) and on drug-dependent antibodies that destroy blood cells.

ITP:

We sought identification of epitopes responsible for reactions with glycoprotein Ib/IX, a major target of ITP Abs, by cloning cDNA fragments of the external domain of GPIbα. Proteins expressed in E.coli DH5a cells reacted with ITP Abs. Short peptides within the large reactive protein fragments, chosen for their likelihood of antigenicity, were synthesized. Reactive epitope(s) for six serum Abs was preserved in one peptide spanning amino acids 326 to 346. Studies such as this one offer insight into the pathophysiology of ITP needed to permit design of more specific therapies.

Our previous studies indicated that sera from a high percentage of patients with ITP contain autoAbs against external and/or internal epitopes on one or more of several platelet membrane glycoproteins. Reports of others suggest that detection of platelet-associated rather than serum Abs against these GPs is the most sensitive and specific test for ITP. This year we found that eluted platelet-associated Abs did not differ from plasma Abs in the degree of reactivity. For clinical applications, tests utilizing serum are much more practical than those utilizing platelets.

Drug Abs:

Hemolytic anemias caused by drug-induced Abs are rare but often fatal disorders. Why certain drug Abs target red cells is a question of basic immunologic and physiologic interest. We found this year that 3 hemolytic drug Abs elicited by dissimilar drugs all were dependent on the erytrocyte integral membrane protein, band 3, for reaction. This is the first identification of the specific red cell component involved. Qualitative and quantitative characteristics of band 3 reactions were defined.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 51, 001-36

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Study of Blood Coagulation and Diseases of Hemorrhage and Thrombosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	N. Raphael Shulman	Chief	CHB:NIDDK
Others:	Diane Reid	Expert	CHB:NIDDK
	Ruyi He	Visiting Associate	CHB:NIDDK
	Junichi Honda	Special Volunteer	CHB:NIDDK
	Charles E. Jones	Chemist	CHB:NIDDK

COOPERATING UNITS (if any)
Charles Carter (CC-DTM); Timothy Maugel, University of Maryland

LAB/BRANCH Clinical Hematology Branch

SECTION

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2.0 PROFESSIONAL: 1.5 OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Platelet membrane glycoproteins (GPs) that act as integrins are necessary for the hemostatic function of platelets. Abnormalities of these GPs are associated with hemorrhage and changes in their activation state may be associated with thrombosis.

Glanzmann's thrombasthenia (GT) is a rare autosomal recessive hemostatic disorder in which platelets do not aggregate in response to physiologic agonists because the platelet membrane integrin αIIbβIIIa (GPIIb/IIIa) is deficient or dysfunctional. The patient studied has markedly reduced quantities of normal-length GP IIb cDNA and an alternatively spliced GPIIb cDNA product in which a partial deletion of exon 28 has been identified. Genomic DNA had no defects in exon 28 or in flanking introns to explain this unusual splicing. Furthermore, there were no sequence defects in the GP IIb promoter region that might have explained the overall reduction of GP IIb mRNA. Normal individuals are currently being screened to determine whether the alternatively spliced product is a polymorphism or unique to this patient.

Platelets vesiculate to produce microparticles (MPs) when activated. MPs express activated integrins which may play a role in hemostasis or thrombosis. Last year we found MPs adsorb to platelets to transfer integrin-associated alloantigens reponsible for posttransfusion purpura. This year we determined the physico-chemical and electron microscopic morphologic characteristics of MPs that support their adsorption to intact platelets. In animal experiments we measured intravascular survival of MP components and their organ localization after leaving the circulation under different physiologic circumstances.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 43002-28 MD

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure, Secretion and Mechanism of Action of Parathyroid Hormone

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.M. Spiegel, M.D.	Chief	MDB, NIDDK
Others:	S.J. Marx, M.D.	Chief, Genet. & Endocrinol. Sec.	MDB, NIDDK
	A.L. Burns	Biologist	MDB, NIDDK
	M.B. Kester	Biologist	MDB, NIDDK

COOPERATING UNITS *(if any)*
Endocrine Unit, Massachusetts General Hospital
National Institute of Dental Research, BRB

LAB/BRANCH
Metabolic Disease Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	3	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 43003-28 MD

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Mode of Action of Thyrocalcitonin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.J. Marx, M.D.	Chief, Genet. & Endocrinol. Sec.	MDB, NIDDK
Others:	J. Barsony, M.D.	Visiting Scientist,	MDB, NIDDK

COOPERATING UNITS *(If any)*

LAB/BRANCH
Metabolic Disease Branch

SECTION
Genetics and Endocrinology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 0.8	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43006-18 MD

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Hyperparathyroidism: Etiology, Diagnosis and Treatment

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.J. Marx, M.D.	Chief, Genet. & Endocrinol. Sec.	MDB, NIDDK
Others:		
A. Spiegel, M.D.	Chief,	MDB, NIDDK
W. McKoy	Chemist	MDB, NIDDK

COOPERATING UNITS *(if any)*

Radiology Department, CC: (J. Doppman, T. Shawker)
Sugery Branch, NCI: (DL Graker, R. Alexander)
Department of Endocrinology, Univ. of Florence, Italy (ML Brandi)

LAB/BRANCH

Metabolic Disease Branch

SECTION

Genetics and Endocrinology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project goal is the evaluation and treatment of hyperparathyroidism. Patients with persistent or recurrent hyperparathyroidism are referred for evaluation and treatment. Hereditary hyperparathyroidism in particular is under investigation in the hopes of delineating hereditary molecular abnormalities in glandular regulation, as exemplified in the multiple endocrine neoplasia syndromes. Evaluation ranges from epidemiologic studies of families to in-house clinical studies of patients and to in vitro analyses of excised tissue. Techniques currently being employed and improved include radioimmunoassay of parathyroid hormone, ultrasonography, (reoperative and intra operative) radiothallium scanning, magnetic resonance imaging, CAT scanning, selective arteriograpy and selective venous sampling for parathyroid hormone, parathyroid gland cryopreservation and autotransplantation, and transcatheter parathyroid gland infarction. In vitro evaluation of parathyroid and other endocrine tissue involves tissue culture, chemistry and determination of linkage with DNA or RNA probes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 43008-12 MD

Period Overed
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Vitamin D Resistance and Related Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.J. Marx, M.D.	Chief, Genetics and Endocriol. Sec	MDB, NIDDK
Others:	J. Barsony, M.D.	Visiting Scientist	MDB, NIDDK,
	W. McKoy	Chemist	MDB, NIDDK
	M. Liberman, Ph.D.	Visiting Fellow	MDB, NIDDK

COOPERATING UNITS *(if any)*
Metabolism Unit, Beilinson Hospital, Betah Tiva, Isreal (U. Liberman)
Hormone Action and Oncogenesis Section, NCI (G. Hager)

LAB/BRANCH
Metabolic Disease Branch

SECTION
Genetics and Endocrinology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

With recognition that vitamin D is the precursor for 1,25-dihydroxyvitamin D, it has become possible to characterize defects in the activation (1-hydroxylation) of vitamin D and defects in the target action of activated (1,25-dihydroxy)vitamin D. We have demonstrated a broad spectrum of manifestations of hereditary resistance to 1,25(OH)2D ranging from infantile rickets with alopecia and no intestinal response to calciferols to adult onset osteomalacia with satisfactory intestinal response to high doses of calciferols and with no epidermal abnormalities. This syndrome usually results from a mutation in the gene for the vitamin D receptor. Skin fibroblasts from all subjects with hereditary resistance to 1,25(OH)2D display abnormalities in this effector system, and defects in many discrete steps of this pathway have been identified with these cells. Cells with mutations in the 1,25(OH)2D effector pathway can be used to explore mechanisms of calciferol action. They have been used to establish that the 1,25(OH)2D receptor mediates an extremely rapid (1-3 minutes) rise of cyclic GMP in response to 1,25(OH)2D3 and that certain receptor mutations compromise many receptor functions but allow another function to be retained normally. This establishes that 1,25(OH)2D receptors couple to different responses by distinct mechanisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 43009-08

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Mineral Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.J. Marx, M.D.	Chief, Genetics & Endocrinol. Sec.	MDB, NIDDK
Others:	W. McKoy	Chemist	MDB, NIDDK
	A. Spiegel, M.D.	Chief,	MDB, NIDDK
	M. Skarulis, M.D.	Senior Staff Fellow	ODIR, NIDDK
	D. Fraker, M.D.		Surg., NCI
	R. Alexander, M.D.		Surg., NCI

COOPERATING UNITS *(if any)*
Bevedere Medical Center - Carisle, PA (J.Green), Dept. of Physiol. Univ. of Manitoba, Canada (J.G. Friesen), Genetics Department - Yale University (A. Bale) Dept. of Endo. - Eniv. of Florence Italy (ML Brandi) - Brigham Hosp. & Harvard

LAB/BRANCH
Metabolic Disease Branch

SECTION
Genetics and Endocrinology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Disorders of mineral metabolism have been evaluated with methods extending from epidemiology to cellular and molecular biology. Two forms of familial hyperparathyroidism have been characterized in detail. Familial hypocalciuric hypercalcemia is an autosomal dominant trait associated with abnormal interactions with calcium in parathyroid and kidney. The affected gene in most kindreds encodes a G-protein linked calcium sensor, and missense mutations have been identified in 3 kindreds. Familial multiple endocrine neoplasia type 1 (FMEN1) is an autosomal dominant trait causing hyperfunction of parathyroids, pancreatic islet and anterior pituitary. It is associated with gradual but abnormal proliferation of the tissues affected. Plasma from affected persons shows high mitogenic activity upon cultured bovine parathyroid cells. This mitogenic activity in plasma may contribute to primary hyperparathyroidism in FMEN1. Analysis of blood and parathyroid tumor DNA has revealed that FMEN1 parathyroids often show clonal loss of alleles in the region of the FMEN1 gene on chromosome 11. Thus the FMEN1 gene probably functions as a tumor suppressor gene, analogous to the retinoblastoma gene. Analysis of sporadic parathyroid adenomas revealed that 25% showed allelic loss in a similar region. Thus the clonal inactivation of the FMEN1 gene may also be a contributing factor in many sporadic parathyroid adenomas. Tumors with allelic loss along chromosome 11 are being used to help delineate the minimal region of allelic loss which must harbor the men1 candidate gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 -DK-43010-01

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Membrane targeting of G proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T. Jones, M.D.	Senior Clinical Investigator, MDB, NIDDK
Others:	M. Degtyarev Visiting	Fellow, MDB, NIDDK

COOPERATING UNITS (if any)
L. Jaffe, Univ. of Conn. Health Science Center, Farmington, CT
P. Backlund, LGCB, NIMH
S. Gutkind, LCDO, NIDR

LAB/BRANCH Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS 2	PROFESSIONAL: 1	OTHER: 1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Heterotrimeric G proteins couple cell surface receptors with intracellular effectors at the cytoplasmic face of membranes. The heterotrimer consists of a subunits which bind the guanine nucleotide and bg subunits which are tightly bound together. The membrane localization of G proteins is critical for their ability to relay signals between membrane-bound receptors and effectors. The a subunits undergo the post-translational modifications of myristoylation and palmitoylation which are important for their membrane attachment. Myristoylation, the addition of a 14 carbon fatty acid to the amino-terminal glycine, is critical for the membrane localization of a subset of a subunits primarily because it increases the affinity of the a subunit for the bg complex. Palmitoylation is the reversible addition of the 16 carbon palmitic acid to cysteine residues through a thioester bond. We found that mutation of the cysteine at residue 3 for as and ai prevents palmitoylation. Activation of the as subunit by isoproterenol or cholera toxin led to an increase in the level of palmitoylation. We found that palmitoylation functions to keep the ai subunit at the membrane when it is free of the bg subunits. Palmitoylation requires that the a subunit is membrane localized but does not require myristoylation. The bg complex can regulate the turnover of palmitate on the a subunits possibly by changing the availability of the cysteine for the modification. These studies on the membrane localization of G proteins enhance our understanding of the regulation and specificity involved in G protein-mediated signal transduction.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-K

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-43011-01

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on a calcium sensing receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Spiegel, M.D.	Chief, MDB, NIDDK
Others:	P. Goldsmith, Ph.D.	Research Biologist, MDB, NIDDK
	A. Varrault, Ph.D.	Special Volunteer
	M. Pena, M.D., Ph.D.	Special Volunteer

COOPERATING UNITS (if any)

Dr. E. Brown, Brigham Hospital, Boston
Dr. F. Fuller , NPS Parmacuticals (proposed CRADA)

LAB/BRANCH Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Brown and colleagues (Nature 1993) have cloned a novel calcium-sensing receptor (CaR) which is a member of the G protein-coupled receptor superfamily similar in structure to the metabotropic glutamate receptors. The CaR is expressed in a limited range of cell types including kidney, brain, thyroid C cells, and most prominently parathyroid cells. Mutations in the CaR identified in the human genetic disorder familial hypocalciuric hypocalcemia provide strong evidence for the physiologic importance of the receptor in calcium sensing generally and in regulation of parathyroid hormone secretion by extracellular ionized calcium in particular. We are studying several aspects of the receptor's structure and function. We have raised polyclonal antisera to several synthetic peptides corresponding to sequences in the large extracellular domain. These recognize the receptor on western blots and should be useful for a wide variety of studies. Development of monoclonal antibodies is planned with the long-range goal of being used as imaging reagents for parathyroid tumors in patients with failed neck explorations. We are also studying the coupling of the receptor to G proteins using transfection techniques, and by defining the normal complement of G protein alpha subunits in parathyroid cells.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-83

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43204-14 MD

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunosuppressive Drug Therapy in Lupus Glomerulonephritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.E. Balow	Senior Investigator	MDB, NIDDK
Others:	H.A. Austin III	Medical Officer	MDB, NIDDK
	D.T. Boumpas	Visiting Scientist	MDB, NIDDK

COOPERATING UNITS *(if any)*

NIAMS (D. Kastner, J. Klippel, P. Plotz, R. Wilder)
CC (E. Vaughan, C. Yarboro)

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20982-1818

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pulse cyclophosphamide is more effective than prednisone alone in preventing renal failure in lupus nephritis. This study sought to define whether pulse methylprednisolone could equal pulse cyclophosphamide in preserving renal function, and whether there was a difference between long and short courses of pulse cyclophosphamide in preventing exacerbations of lupus. Patients were treated with prednisone and randomized to receive concomitantly (a) pulse methylprednisolone monthly for 6 months, or (b) pulse cyclophosphamide monthly for 6 months, or (c) pulse cyclophosphamide monthly for 6 months followed by a maintenance regimen every 3 months for an additional two years. Patients treated with pulse methylprednisolone had a higher probability of developing renal insufficiency than patients treated with the long course of pulse cyclophosphamide. In addition, patients treated with only a short course of pulse cyclophosphamide had a higher probability of major exacerbations of lupus than those treated with the extended course of pulse cyclophosphamide. Studies of ovarian toxicity in patients treated with pulse cyclophosphamide showed that risk is affected by both age at treatment and total doses of cyclophosphamide. A series of clinical and pathologic factors were analyzed for their ability to predict renal failure subsequent to entry into the therapeutic trials. Black race, age >30 years, anemia, elevated serum creatinine and hypocomplementemia significantly predicated adverse renal outcomes. However, renal biopsy features contributed additional prognostic information and significantly enhanced the strongest clinical model.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43205-17 MD

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Renal Biopsy Pathology in Systemic Lupus Erythematosus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.E. Balow	Senior Investigator	MDB, NIDDK
Others:	H.A. Austin III	Medical Officer	MDB, NIDDK

COOPERATING UNITS *(if any)*

Armed Forces Institute of Pathology, Washington, DC (S. Sabnis); CC (E. Vaughan)

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20982

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Diverse pathogenetic factors are operant in systemic lupus erythematosus and lead to different forms of lupus nephritis. Detailed analysis of renal biopsy pathology is being conducted on specimens from patients with systemic lupus erythematosus.

Biopsies are classified by standard major category of lupus nephritis, as well as scored on a semi-quantitative scale for specific histologic changes which indicates the extent and severity of active inflammatory lesions and chronic atrophic, fibrosing and sclerosing features. The patterns of immune complex deposition and lymphoid cell interaction with different segments of the nephron are being investigated by immunohistologic techniques and electron microscopy.

These approaches have facilitated the analysis of the effects of various types of immunosuppressive agents used to halt the progression of lupus nephritis and they will enhance our understanding of the pathogenesis of lupus nephritis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43208-01 MD

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Halogenated Adenosine Analogs as Immunosuppressive Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	D.T. Boumpas	Visiting Scientist	MDB, NIDDK
Others:	B.J. Fessler	Clinical Associate	MDB, NIDDK
	S. Shrivastav	Chemist	MDB, NIDDK
	H.A. Austin	Medical Officer	MDB, NIDDK
	J.E. Balow	Senior Investigator	MDB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH Bethesda, MD 20892-1818

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.9	0.8	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The clinical efficacy, toxicity, and immunological effects of halogenated adenosine analogs, such as fludarabine and chlorodeoxyadenosine, are being studied in patients with autoimmune disorders. These agents primarily affect lymphoid cells because these cells contain enzymes necessary for activation of these compounds. Previous investigations employing these drugs in patients with low grade lymphoid malignancies have shown that they selectively target immune cells by inducing a physiologic form of cell death called apoptosis. Their effects on the clinical course of immunologic diseases, as well as on the function of immune cells have not been characterized.

Five patients have been entered into a study for treatment of refractory membranous nephropathy. To date, no major or unexpected toxicities have been encountered. Fludarabine treatment has resulted in sustained B and T lymphopenia (B>T). We are attempting to determine whether the level of lymphopenia should be a major determinant of safe drug doses; no substantive changes in total immunoglobulin or antibody levels have been seen. At the low doses of fludarabine used to date, no measurable clinical effects have been noted on the nephropathy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 43211-10

PERIOD COVERED
10/1/93 thru 9/30/94

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Morphometry of the Glomerulus in Pimas and other Minority Populations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,
PI: GE Striker, Senior Investigator
Others: LJ Striker, Chief RCBS

COOPERATING UNITS (if any)
A Fogo, Dept. Path, Vanderbilt U, Nashville, TN
C M Pesce University of Genova Italy

LAB/BRANCH
Metabolic Diseases

SECTION
Renal Cell Biology, Bldg. 10, Rm. 3N-110

INSTITUTE AND LOCATION
NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS: .10	PROFESSIONAL: .10	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
(a) Human X (b) Human (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The mean of the area and a size-class distribution of the glomeruli were calculated with a computer-assisted planimeter in a series of autopsy specimens from subjects without any evidence of renal disease. These morphometric variable were also related to the severity of histologic glomerular lesions, graded on a four class scale. Glomerular size was independent of body mass index and kidney weight. This morphometric analysis was compared with that obtained from a series of forensic autopsies from Caucasians matched for age and sex. The mean glomerular volume was greater in the Pima Indians than in the Caucasians. Furthermore the volume fraction of the cortex occupied by the glomeruli was greater in the Pima Indians, suggesting that their increased glomerular volume was not due to a reduced number of glomeruli.

The large size of the glomeruli in the Pima Indians was independent of obesity or heart weight and could constitute a genetic trait of this population. In this population the changes in glomerular mass and filtration surface may underlie a familial predisposition to developing renal disease.

Finally we undertook a similar study in a series of forensic autopsies in African Americans since their incidence of end stage renal disease is much higher than in the caucasian population. The mean size of the glomeruli was significantly greater in the African American population. We postulate that the increased glomerular size may constitute a marker for a sclerosis-prone population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-43214-10 MD

PERIOD COVERED

10/01/93 thru 9/30/94

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Glomerular Cells Derived from Normal and Transgenic Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute

PI: GE Striker, Senior Investigator
Others: LJ Striker, Chief RCBS
Ci-Jiang HE, Visiting Associate
Zhi-Hong Liu, Visiting associate

COOPERATING UNITS (if any)

Phillip Stahl, Washington University
Maria Tsokos, Laboratory of Pathology, NCI

LAB/BRANCH

Metabolic Diseases

SECTION

Renal Cell Biology, Building 10, Room 3N-110

INSTITUTE AND LOCATION

NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.95	0.95	

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues X (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Glomerulosclerosis, characterized by increased glomerular cell turnover and increased turnover of extracellular matrix turnover, is the leading cause of renal failure in the US. Current studies in humans or animal models of glomerulosclerosis (GS) have yielded little information about the cellular and molecular abnormalities that are critical in the initiation and progression of this disease. This is due, in part, to the difficulty in isolating and characterizing glomeruli in vivo, and the fact that the glomerulus contains three indigenous cell types and a population of bone marrow-derived macrophages. This is particularly a problem with mice, a species we have chosen to study because of the availability of considerable genetic information about extracellular matrix and growth regulatory factors. We have approached this problem by isolating the individual cell types and characterizing them in vitro. The first series of experiments examined the question of the phenotypic modulation of glomerular mesangial cells in vitro, addressing the question of the effect of differing substrates, the time frame involved, and the molecular consequences of this modulation on extracellular matrix turnover. We found that mesangial cell proliferation and collagen mRNA levels are independently regulated. In addition, the nature of the matrix to which the cells adhered directly affected both proliferation and collagen mRNA levels. Finally, the presence or absence of adherence was important in determining the proliferation rate. The second experiments addressed the handling of macromolecules by mesangial cells, since these cells are capable of ingesting immunoglobulins in vivo. The mannose receptor (MR), a carbohydrate binding membrane protein present on macrophages, was considered to be a candidate for the mechanism by which mesangial cells ingested these large proteins. The MR was found on mesangial cells which had been stimulated with either interleukin-1α or tumor necrosis factor-α.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43222-09 MD

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Murine Lupus Nephritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.:	H. A. Austin III	Medical Officer	MDB, NIDDK
Others:	J. E. Balow	Senior Investigator	MDB, NIDDK
	D. T. Boumpas	Visiting Scientist	MDB, NIDDK

COOPERATING UNITS *(if any)*

Armed Forces Institute of Pathology; Washington, DC (S. Sabnis)

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892-1818

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigations of the pathogenesis and treatment of lupus nephritis are facilitated by the availability of inbred strains of mice that develop disease similar to human systemic lupus erythematosus. The natural evolution of the diverse histologic features of murine lupus nephritis has been studied to delineate the types of glomerular and tubulointerstitial lesions.

Innovative treatment strategies will be studied to refine our approach to this disease. The impact of biologic response modifiers on immunologic features will be investigated as well. Clinical, histologic and immunologic outcome parameters will be evaluated including detailed studies of renal morphology, and the characteristics of spleen lymphocytes employing flow cytometry, measures of immunoglobulin gene expression, and in vitro assays of alterations in humoral and cell mediated immune regulation.

An investigation of the effects of a monthly doses of intraperitoneal cyclophosphamide in NZB/W female mice has been undertaken. Multiple doses of "pulse" cyclophosphamide lead to prolonged effects on the numbers (total and phenotypes) and the functions (spontaneous immunoglobulin secreting cell responses) of lymphocytes (spleen and peripheral blood). Additional studies regarding the mechanisms underlying these effects are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43224-08 MD

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Membranous Lupus Nephropathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. A. Austin III	Medical Officer	MDB, NIDDK
Others:	J. E. Balow	Senior Investigator	MDB, NIDDK

COOPERATING UNITS *(if any)*

CC (E. Vaughan); Stanford University; Stanford, CA (B. Myers); Armed Forces Institute of Pathology, Washington, DC (S. Sabnis)

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892-1818

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is currently unknown whether therapeutic intervention will alter the course of membranous lupus nephropathy. In the present study, the efficacy and toxicity of three immunosuppressive drug regimens administered over a 12 month period will be evaluated in patients with membranous lupus nephropathy. Detailed tests of renal function (including radiolabelled compounds for glomerular filtration and renal plasma flow rates), glomerular permselectivity (using fractional clearance of graded dextrans) and kidney biopsy morphology will be performed at the beginning and end of treatment. Patients with systemic lupus erythematosus, nephrotic range proteinuria and biopsy documented membranous nephropathy will be randomized to receive: a) alternate day prednisone alone (control group), b) alternate day prednisone plus intravenous pulse cyclophosphamide up to 1.0 gram per square meter body surface area every other month for 6 total doses, or c) alternate day prednisone plus oral cyclosporin A up to 200 mg per square meter body surface area daily. Lupus disease activity, renal function tests and drug toxicities will be monitored closely. Analysis will include comparison of the numbers of favorable outcomes of glomerular filtration rate, renal plasma flow, permselectivity, glomerular pathology and drug-related toxicities appearing in each treatment group. To date, response rates have been higher in patients receiving cyclophosphamide or cyclosporin A than in those receiving corticosteroids alone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 43225-07

PERIOD COVERED
10/1/92 thru 9/30/93

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Glomerular Lesions in Mice Transgenic for Growth Hormone

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute
PI: LJ Striker, Chief RCBS
Others: GE Striker, Senior Investigator
EP Peten, Visiting Associate
CW Yang, Visiting Associate

COOPERATING UNITS (if any)
John Kopchick, University of Ohio

LAB/BRANCH
Metabolic Diseases

SECTION
Renal Cell Biology, Bldg. 10, Rm. 3N-110

INSTITUTE AND LOCATION
NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
(a) Human subjects (b) Human tissues X (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have shown that mice transgenic for growth hormone develop a rapidly progressive glomerulosclerosis. Mice transgenic for GH molecules containing mutations have also been examined in order to dissociate the domains of GH that may specifically code for genes responsible for glomerulosclerosis from those coding for body mass increase. We found an upregulation of mRNA for extracellular matrix components in the glomeruli of the GH animals by competitive PCR of isolated microdissected glomeruli. This upregulation persisted late in the course of the disease. We have started exploring therapeutic manipulations using angiotensin converting enzyme inhibitors (ACEi) and non-anticoagulant heparin. The effects of treatment were followed by morphology, and competitive PCR for type IV collagen, type I collagen, smooth muscle actin, and laminin B1 mRNAs. The course of the glomerular lesions was unaltered following ACEi therapy and there appeared to be a worsening of vascular lesions, presumably due to a local increase of renin. In addition, there was no decrease in mRNAs coding for any of the genes listed above. In contrast, the lesions were reduced by light microscopy and the mRNA coding for the 92kD matrix metalloproteinase complex was increased after two weeks of heparin treatment. The nature of the heparin effect is currently being investigated in further detail, since this molecule or other related species, could be used in the treatment of intractable progressive human glomerulosclerosis. Mice transgenic for a mutated growth hormone (M11) exhibit a much slower rate of progression than the mice described above. The levels of glomerular ECM mRNAs, measured early at 3 months of age, are intermediate between those of the GH mice and normal controls. These data suggest that fast and slow progressors can be identified prior to the appearance of histologic lesions, which had heretofore had been the sole means to determine the presence of glomerulosclerosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 43227-07 MD

PERIOD COVERED
10/1/93 thru 9/30/94

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Role of IGF-I in the Biology of Mesangial Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute

PI: LJ Striker, Chief RCBS
Others: G Striker, Senior Investigator
S Elliot GS12
Ci-Jiang He, Visiting Associate
EP Peten, Visiting Associate

COOPERATING UNITS (if any)
M Hattori, Joslin Institute, Harvard Medical School, Boston.

LAB/BRANCH
Metabolic Diseases Branch

SECTION
Renal Cell Biology

INSTITUTE AND LOCATION
NIDDK, Bldg 10, Room 3N-110 Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	

CHECK APPROPRIATE BOX(ES)
(a) Human subjects (b) Human tissues X (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 43228-07 MD

PERIOD COVERED
10/1/92 thru 9/30/93

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biology of Human Mesangial Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute
PI: GE Striker, Senior Investigator
Others: LJ Striker, Chief RCBS

COOPERATING UNITS (if any)

LAB/BRANCH
Metabolic Diseases

SECTION
Renal Cell Biology

INSTITUTE AND LOCATION
NIDDK NIH Bethesda, Md 20892, Bldg 10 Room 3N-110

TOTAL STAFF YEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
(a) Human subjects X (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is currently inactive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43231-06 MD

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Idiopathic Membranous Nephropathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	H. A. Austin III	Medical Officer	MDB, NIDDK
Others:	J. E. Balow	Senior Investigator	MDB, NIDDK

COOPERATING UNITS *(if any)*

Stanford University, Stanford, CA (B. Myers); CC (E. Vaughan); Armed Forces Institute of Pathology; Washington, DC (S. Sabnis)

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892-1818

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with idiopathic membranous nephropathy are being studied to evaluate the efficacy and toxicities of the addition of intermittent cyclophosphamide to alternate day oral corticosteroid therapy. Efficacy will be judged by determinations of effective renal plasma flow, glomerular filtration rate and glomerular capillary wall permselectivity performed with dextran and urine protein (albumin and immunoglobulin) clearance techniques. Kidney biopsy morphology (including morphometric analysis) will be examined at the beginning and at the end of treatment as part of detailed studies of structure-function relationships and the efficacy of various therapeutic modalities.

Patients with membranous nephropathy and 2 or more grams per day of proteinuria will be treated with alternate day prednisone and will be randomized to receive: a) no additional therapy (control group), or b) intravenous pulse cyclophosphamide up to 1.0 gram per square meter body surface area every other month for 6 total doses. Analysis will include comparison of the number of favorable outcomes of glomerular function and pathology, as well as drug-related toxicities observed in each treatment group at the end of the 12 months of study.

To date, the rates of renal outcomes are not significantly different in the groups receiving prednisone alone or prednisone plus intermittent cyclophosphamide.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 43232-05 MD

PERIOD COVERED
10/1/93 thru 9/30/94

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Renal Lesions in the Ablation Model

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute
PI: LJ Striker, Chief RCBS
Others: GE Striker, Senior Investigator

COOPERATING UNITS (if any)
Saulo Klahr, Washington University, St Louis, MO
Carlo Pesce, Dept of Pathology, Genova, Italy

LAB/BRANCH
Metabolic Diseases

SECTION
Renal Cell Biology

INSTITUTE AND LOCATION
NIDDK Bethesda MD 20892, Bldg 10, Room 3N-110

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects (b) Human tissues X (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Subtotal nephrectomy in rats, the 5/6ths ablation model, leads to progressive loss of kidney function and chronic renal failure. We postulated that the progressive glomerular lesions were due to an early increase in the turnover of glomerular resident cells. This leads to abnormal glomerular growth, with an increase in the glomerular volume which was detectable using morphometric measurements. We have performed autoradiographic studies, using ^{3}H-thymidine, and found that within two days following subtotal nephrectomy there was an increase in the glomerular cell mitotic index, as well as an increase in the turnover of the cells in the arterial wall. These findings suggest that dysregulation of cell growth is an early event in the development of glomerulosclerosis following reduction in renal mass.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43234-05 MD

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interactions Between TGF-β and Glomeruli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	K. MacKay	Expert	MDB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892-1818

TOTAL STAFF YEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43235-05 MD

PERIOD COVERED

10/1/93 thru 9/30/94

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Glomerular Lesions in Non-Obese Diabetic Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: LJ Striker, Chief RCBS
Others: GE Striker, Senior Investigator
C W Yang, Visiting Associate
Ci Jiang He, Visiting Associate
Zhi Hong Liu, Special Volunteer

COOPERATING UNITS (if any)

M Hattori, Joslin Institute, Harvard Medical School, Boston, MA

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Renal Cell Biology

INSTITUTE AND LOCATION

NIDDK, Bldg 10, Room 3N-110, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.60	0.60	

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues X (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

NOD mice spontaneously develop insulin-dependent diabetes mellitus (IDDM) secondary to immunologically-mediated beta cell destruction in pancreatic islets. These mice exhibit an increase in the amount of mesangial matrix before diabetes mellitus occurs and are therefore "prone to glomerulosclerosis". Shortly after the appearance of diabetes, there is an accentuation of the renal lesions consisting of mesangial sclerosis, thickening of glomerular basement membranes, and albuminuria. Morphometric analysis showed that the kidney weight and glomerular size were increased in diabetic mice, compared to non-diabetic mice, and that the ratio glomerular volume/kidney weight was elevated in diabetic mice. We explored by competitive PCR of isolated glomeruli the glomerular turnover of the extracellular matrix components. Prior to the appearance of diabetes the levels of type IV collagen mRNA were 3-4 times higher than in SJL mice who have normal glomeruli. In addition the NOD mice had fewer glomeruli than the SLJ and those were larger. Therefore the NOD strain has a propensity to develop sclerosis irrespective of diabetes which is underlied by an abnorma level of expression of basement membrane gene expression and by a reduced number of glomeruli. Shortly after the occurence of diabetes mellitus we found an upregulation of laminin but not collagens gene expression. This was associated with an increase in TGF-ß1 gene expression which may represent one of the early molecular events in diabetic nephropathy. We also found that there was accumulation of the advanced glycosylation end products in the kidneys shortly after the onset of diabetes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43236-04 MD

PERIOD COVERED

10/1/93 thru 9/30/94

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Glomerular Effects of Advanced Glycosylation End Products(AGEs)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: LJ Striker, Chief RCBS
Others: CW Yang, Visiting associate
GE Striker, Senior investigator

COOPERATING UNITS (if any)

Helen Vlassara, Picower Institute for Medical Research, Manhasset NY

LAB/BRANCH

Metabolic Diseases

SECTION

Renal Cell Biology

INSTITUTE AND LOCATION

NIDDK, Bdg 10, Room 3N-110, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.55	0.55	

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues X (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

End-stage glomerulosclerosis constitutes a major complication of diabetes mellitus. The fact that the glomerular lesions in both IDDM and NIDDM are similar suggests that abnormalities in glucose metabolism may participate in their development. Hyperglycemia leads to the accumulation of advanced glycosylation end-products. These products participate in abnormal, non-metabolizable cross-linking of extracellular matrix components. Their accumulation may contribute to the sclerosis observed in diabetics. AGEs trigger a large number of biological reactions which are mediated by surface receptors that have been characterized on macrophages, endothelial cells, and human and rat mesangial cells. Using normal mouse mesangial cells, we showed that cells exposed to AGE had increased levels of the following mRNAs using the RNAse protection assay: collagen type IV, proteoglycan heparan sulfate, and laminin A and B chains. We also found an increased release of collagen type IV into the medium. We explored the effects of injections of AGEs to to the intact animal would have similar effects. normal rats receiving long-term injections of AGE-Albumin had albuminuria and developed focal sclerotic glomerular lesions. The coadministration of aminoguanidine which inhibits crosslinking of AGEs prevented the renal lesions. We also found that the glomeruli of normal mice receiving repeated injections of AGEs exhibited an increase in mRNAs coding for α1 type IV collagen and for the B1 chain of laminin establishing that there is a glomerular response to AGEs in vivo. These responses were associated with an increase in TGF-β1 but not PDGF message level. These responses did not occur when the mice were cotreated with aminoguanidine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43237-03

PERIOD COVERED

10/01/92 thru 9/30/93

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Production of Metalloproteinases and TIMPs by Mouse Glomerular Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute

PI: LJ Striker, Chief RCBS
Others: G Striker, Senior Investigator
S. Elliot

COOPERATING UNITS (if any)

W Stetler-Stevenson, Laboratory of Pathology, NCI, NIH
M Carome, Walter Reed Army Medical Center

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Renal Cell Biology

INSTITUTE AND LOCATION

NIDDK, Bldg. 10, Rm. 3N-110, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.08	0.07	0.01

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues X (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Glomerulosclerosis occurs in a large number of human kidney diseases, including diabetic nephropathy. It consists of the accumulation of extracellular matrix (ECM) within the glomerulus. Sclerosis may be mediated by dysregulation of both synthesis and degradation of ECM. A large family of matrix metalloproteinases as well as tissue inhibitors of metalloproteinase (TIMPs) play a role in the degradative process. Our preliminary work reveals that: 1) normal mouse mesangial cells in culture secrete a 72 kD and a 92 kD gelatinase (type IV collagenase) as well as TIMP1; 2) mesangial cells derived from NOD mice and mice transgenic for bovine growth hormone (bGH) secrete mostly the 72 kD gelatinase; Normal mouse glomeruli contain mRNA coding the 72KD species. This mRNA is increased in mice transgenic for bGH who have sclerotic lesions.TIMPs mRNAs are undectable in mouse glomeruli which emphasizes the major phenotypic changes that occur in vivo. In the GH mice the upregulation of the 72KD gelatinase is less prominent than that of type IV collagen. This finding may suggest that there is an imbalance between synthesis and degradation of extracellular matrix in certain forms of glomerulosclerosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43238-04 MD

PERIOD COVERED

10/01/93 thru 9/30/94

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Expression in Microdissected Mouse Glomeruli

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: L Striker, Chief RCBS
Others: G Striker, Senior Investigator
E Peten, Visiting Associate
CJ He, Visiting Associate
Zhi-Hong Liu

COOPERATING UNITS (if any)

R Zalups PhD, Department of physiology , Macon Georgia

LAB/BRANCH

Metabolic Diseases

SECTION

Renal Cell Biology

INSTITUTE AND LOCATION

NIDDK, Bldg 10, Room 3N-110, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.47	0.47	

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues X (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is to determine the phenotype of multiple extracellular matrix molecules in adult mouse glomeruli from different strains of mice. our postulate is that mice prone to develop glomerulosclerosis have a higher level of expression of genes coding for extracellular matrix at baseline prior to the appearance of lesions. We used the increased sensitivity afforded by the polymerase-chain reaction to assess α1 type I and several α chains of type IV collagen mRNA as well as laminin subchains in freshly microdissected normal adult mouse glomeruli. RT-PCR reactions for mRNA encoding these components were also performed using mesangial cell lines previously isolated from the same strain of mice. Type IV collagen mRNA was easily detectable in normal adult mouse glomeruli as well as in the cell lines. On the other hand, type I collagen mRNA was not detected in normal glomeruli, despite increasing the number of PCR cycles from 25-45 (roughly a 1000 fold increase in sensitivity). Assays using competitive PCR were developed. The current study provides evidence that the expression of types I and IV collagen in normal glomeruli is regulated at the pretranslational level in vivo. They also provide evidence for a continuous turnover of basement membrane in the adult glomeruli. We have applied this method to a mouse strain which develops spontaneous glomerulosclerosis, the ROP-OS mouse. This mouse is originally derived from a radiation-induced mutation. Our preliminary data indicate that the mutation induces a rapid glomerulosclerosis on a ROP background but has little effect on a C a C57B background. The ROP-OS mouse has a 3 fold increase in mRNAs coding for type IV collagens and laminin early in life at a time when morphologic evidence of glomerulosclerosis is quite discrete.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43239-03 MD

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms and Modulation of T cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.:	D.T. Boumpas	Visiting Scientist	MDB, NIDDK
Others:	N. Hama	Visiting Fellow	MDB, NIDDK
	F. Paliogianni	Courtesy Associate	MDB, NIDDK
	B.J. Fessler	Clinical Associate	MDB, NIDDK
	S.S. Ahuja	Visiting Associate	MDB, NIDDK
	S. Shrivastav	Chemist	MDB, NIDDK
	J.E. Balow	Senior Investigator	MDB, NIDDK

COOPERATING UNITS *(if any)*

University of Washington, Seattle (M.A. Valentine)
Section on Immunology, NIAAA, NIH (R.L. Kincaid)

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892-1818

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.3	2.4	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Activation of T cells is a complex process involving cell membrane, cytoplasmic and nuclear events. These events enable T cells to proliferate and exert their immunoregulatory function.

The purpose of these studies is to define the effects of biologic agents used for the therapy of immune mediated renal diseases (glucocorticoids, cyclosporine A), as well as those produced at the site of inflammation (prostaglandin E_2, transforming growth factor-β), on molecular events associated with T cell activation. T cell activation through its antigen specific receptor results in increased intracellular calcium and activation of protein kinase C (PKC). Increased intracellular calcium, in turn, activates calmodulin (CaM) dependent kinases, such as CaM kinase II and phosphatases, such as calcineurin.

Glucocorticoids downregulate the activity of CaM kinase II, but not PKC, during T cell activation by upregulating protein phosphatases 1 and/or 2A, but not 2B. Both cyclic AMP elevating agents and glucocorticoids inhibit calcineurin-dependent pathways for T cell activation (calcineurin being a calcium/calmodulin-dependent 2B phosphatase).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 43240-03 MD

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transcriptional Regulation of Immunoglobulin Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: D.T. Boumpas Visiting Scientist MDB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892-1818

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 43241-03

PERIOD COVERED
10/01/93 thru 9/30/94

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of the Pathogenesis of Glomerulosclerosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute*
PI: LJ Striker, Chief RCBS
Others: GE Striker, Senior Investigator
EP Peten, Visiting Associate
CJ He, Visiting Associate
Zhi Hong Liu, Special Volunteer

COOPERATING UNITS *(if any)*
M Carome, Walter Reed Army Medical Center
International Collaborative Group for Molecular Studies of Kidney Biopsy (See list below)

LAB/BRANCH
Metabolic Diseases

SECTION
Renal Cell Biology

INSTITUTE AND LOCATION
NIDDK, Bldg. 10, Rm. 3N-110, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.65	PROFESSIONAL: 0.65	OTHER:

CHECK APPROPRIATE BOX(ES)
☐(a) Human X ☐(b) Human ☐(c) Neither
☐(a1) Minors
☐(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Most progressive glomerular diseases leading to endstage renal disease have increased extracellular matrix (ECM) within mesangial areas. Elucidation of the nature and the rate of ECM accumulation in normal and diseased states are prerequisites for understanding, and possibly interfering with, progressive glomerulosclerosis. This was the rationale to undertake detailed studies addressing these issues in normal subjects and patients with progressive glomerular diseases. Glomeruli represent a small fraction of the renal cortex and we have found that changes in their size and matrix synthesis are regulated independently of other cortical elements. Therefore, changes in whole kidney preparations do not accurately reflect those in glomeruli. For these reasons we developed a technique consisting of microdissection of glomeruli, in situ RT of mRNA, and PCR. To quantitate changes in type IV collagen mRNA expression, we developed a competitive PCR assay. The method has a level of sensitivity (0.01 to 0.1 attomole) sufficient to allow quantitation of the α2IV collagen cDNA expressed in a fraction of a mouse glomerulus. This allowed us to begin studies of human glomerular diseases. We first examined nephrectomies performed for renal carcinoma, since nearly 50% of these patients have glomerulosclerosis. We found that α2IV collagen cDNA was significantly elevated in patients with glomerulosclerosis. There was not a parallel increase in cell number, suggesting that the increment was due to upregulation of α2IV collagen mRNA, rather than cellular proliferation. As we found in mice, glomerular α2IV collagen cDNA level remained high, independently of the degree of sclerosis, even in patients who had extensive glomerular scarring. Satisfied that we had the appropriate tools to examine the pathogenesis of renal disease in humans, we started a multi-center collaboration to develop markers of progression. During this first phase we obtained either cDNA or isolated microdissected glomeruli from renal biopsies done for diagnostic purposes. The initial focus is on diabetes mellitus and IgA nephropathy. Our preliminary results indicate that the α2/α3 type IV collagen ration is equal to 1 in normal kidneys.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 43242-03 MD

PERIOD COVERED
10/1/93 thru 9/39/94

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Degradation of Extracellular Matrix in Human Glomeruli

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: LJ Striker, Chief RCBS
Others: GE Striker, Senior Investigator
EP Peten, Visiting Associate

COOPERATING UNITS (if any)
MA Carome, Walter Reed Army Medical Center
WG Stetler-Stevenson, NCI, Laboratory of Pathology

LAB/BRANCH
Metabolic Diseases

SECTION
Renal Cell Biology

INSTITUTE AND LOCATION
NIDDK, NIH Bdg 10 Room 3 N110 Bethesda MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.21	0.21	

CHECK APPROPRIATE BOX(ES)
(a) Human subjects X (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Glomerulosclerosis is characterized by a net accumulation of extracellular matrix components in the glomerulus and results in its progressive obliteration. We started to investigate whether this condition could result from an imbalance between synthesis and degradation of the extracellular matrix. The metalloproteinases which degrade basement membrane collagens are inactivated by a series of inhibitors: TIMPs (tissue inhibitors of metalloproteinases). We examined whether TIMPs mRNAs and the corresponding peptide were expressed in normal and sclerotic glomeruli. We utilized the non tumoral part of nephrectomies performed for cancer in a series of 10 adult patients. The glomeruli were microdissected and reverse-transcribed in situ. Competitive polymerase chain reaction was performed for TIMP I and II mRNAs. Both were detected in the glomeruli and were increased in in the specimens in which glomerulosclerosis was present. The increase was not due to an increase in the number of cells in the glomeruli with sclerosis. These data suggest that TIMPs are increased on a per cell basis in the glomerulosclerosis associated with carcinoma. Further studies are in progress to determine whether this increase is also observed in other forms of human glomerulosclerosis. We have also obtained preliminary data indicating that mRNA coding for the 72kd metalloproteinase is expressed in human glomeruli. This species appears to undergo the same upregulation than the $\alpha 2$ chain of type IV collagen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43243-02 MD

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Co-stimulatory Signals for T-cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	D.T. Boumpas	Visiting Scientist	MDB, NIDDK
Others:	B.J. Fessler	Clinical Associate	MDB, NIDDK
	F. Paliogianni	Courtesy Associate	MDB, NIDDK
	N. Hama	Visiting Fellow	MDB, NIDDK
	J.E. Balow	Senior Investigator	MDB, NIDDK

COOPERATING UNITS *(if any)*

Laboratory of Experimental Immunology, FCRP, NCI (H.A. Young)

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892-1818

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Molecules that interact with the antigen-specific receptor on T-cells, including peptides bound to major histocompatibility complex (MHC) molecules, antibodies against the antigen specific receptor, and mitogenic lectin, fail on their own to stimulate T cells to proliferate. At least one additional signal is required. This second signal is referred to as co-stimulation, and it is delivered by antigen-presenting cells.

The molecular nature of co-stimulation has been sought for many years in order to facilitate therapeutic strategies for suppressing immune responses. The goal of these studies is to dissect further the molecular mechanisms of co-stimulation by the use of naturally occurring immunosuppressive agents.

Findings to date indicate that glucocorticoids inhibit activation of NF-AT and IL-2Rα expression but not the tyrosine phosphorylation and calcium influx when cells are stimulated via CD2. IL-2 production is inhibited by glucocorticoids in cells treated with phorbol ester and anti-CD28.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43300-01 MDB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular biologic studies on the cause of parathyroid neoplasia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Spiegel, M.D.	Chief, MDB, NIDDK
Others:	D. Wenkert, M.D.	Clinical Associate, MDB, NIDDK
	Q. Dong, M.D., Ph.D.	Visting Fellow, MDB, NIDDK

COOPERATING UNITS (if any)

S. Marx, M.D., Chief, Genetics and Endocrinology Section, MDB, NIDDK

LAB/BRANCH Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS .25	PROFESSIONAL: .25	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Primary hyperparathyroidism (HPT), a common endocrine disorder that can cause significant morbidity, may be due to benign neoplasia of a single parathyroid gland (adenoma) or multiple parathyroid glands (hyperplasia), and rarely, to malignant neoplasia of a parathyroid gland (carcinoma). The etiology of parathyroid neoplasia has not been defined. As with other forms of neoplasia, parathyroid tumors are presumably due to inherited (germ-line mutation) and/or acquired (somatic mutation) defects in specific genes. Etiologic genetic defects could include inappropriate expression of transforming "oncogenes" and/or loss of expression of tumor "suppressor" genes. The availability of surgically resected parathyroid tumors from patients with sporadic and hereditary forms of disease allows us to search for tumor-specific genetic abnormalities that may be involved in development of parathyroid neoplasia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DK 43301-01 MDB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Guanine nucleotide binding proteins as receptor-effector couplers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Spiegel, M.D.	Chief, MDB, NIDDK
Others:	A. Shenker, M.D.	Senior Res. Inv., MDB, NIDDK
	Q. Dong, M.D., Ph.D.	Visiting Fellow
	P. Goldsmith, Ph.D.	Research Biologist, MDB, NIDDK

COOPERATING UNITS (if any)
C. Unson, Rockefeller Univ., N.Y.
J. Feramisco, UCSD, LA Jolla, CA

LAB/BRANCH Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS 2.5	PROFESSIONAL: 2	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A family of guanine nucleotide binding proteins (G-proteins) functions in transmembrane signalling as receptor-effector couplers. G-proteins couple to a diverse array of receptors including those for hormones, neurotransmitters, light, odorants, and certain growth factors. Effector functions regulated (positively and, in some instances, negatively) by G-proteins include cAMP formation, phosphoinositide breakdown, potassium and calcium channels, and cGMP degradation. We have used a variety of techniques to study the expression, distribution, regulation, structure and function of G-proteins. Our studies highlight the diversity within the G-protein family. Using peptide specific antibodies, we have defined the specificity of G-proteins in coupling to receptors and effectors. We have created mutations in alpha subunits that cause constitutive activation, and transfected these into cells to define phenotypic effects on cellular function. We have identified mutations in G proteins and in G protein-coupled receptors as the basis of several human diseases. These studies provide the basis for understanding the role of G-proteins in normal signal transduction and for elucidating possible defects in G-protein structure or function as the basis for abnormal signal transduction.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-80

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 43302-01 MDB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on pseudohypoparathyroidism and related disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. Weinstein, M.D.	Senior Staff Fellow, NIDDK, MDB

Others:

S.-H. Yu, Ph.D.	Visiting Associate, MDB, NIDDK
D. Yu, Ph.D.	IRTA, MDB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH Metabolic Diseases Branch

SECTION Molecular Pathophysiology Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS 2.5	PROFESSIONAL: 2	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In 1942 Albright described the features of an inherited clinical syndrome named "pseudohypoparathyroidism" (PHP). The biochemical hallmark of PHP is a lack of response of urinary cyclic AMP (cAMP) to exogenous parathyroid hormone (PTH). This indicates that there is a defective hormone receptor-adenylyl cyclase complex in this disorder. Patients with one form of PHP show characteristic constitutional features (Albright's hereditary osteodystrophy - AHO) and resistance to multiple hormones including PTH,thyrotropin and gonadotropins (PHP Ia). Other affected relatives from PHP Ia kindreds have AHO without hormone resistance (pseudopseudo-hypoparathyroidism, PPHP). We have shown that many patients with PHP Ia and PPHP show an approximately 50% reduction in activity of Gs (the stimulatory guanine nucleotide binding protein associated with adenylyl cyclase) in membranes from multiple tissues. We have shown that steady state Gs-alpha mRNA levels from fibroblasts of subjects with PHP Ia and PPHP are reduced by approximately 50% compared with normals and have identified genetic abnormalities which account for Gs deficiency. It has recently been suggested that the same genetic defect in the Gs-alpha gene may result in PHP Ia or PPHP by a mechanism called parental imprinting. Another form of PHP is characterized by isolated PTH resistance without features of AHO or resistance to other hormones (PHP Ib). Previous work in this laboratory showed a selective resistance of cAMP generation to PTH in fibroblasts from these patients, suggesting a potential defect in the PTH receptor. Molecular studies to date suggest that these are not due to mutations in the coding exons of the PTH receptor gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 43303-01 MDB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on McCune-Albright Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Spiegel, M.D.	Chief, MDB, NIDDK
Others:	L. Weinstein, M.D.	Senior Staff Fellow, MDB, NIDDK
	A. Shenker, M.D, Ph, D.	Senior Research Inv., MDB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

McCune-Albright syndrome (MAS) is a non-genetic disorder in which affected subjects show a variety of seemingly unrelated abnormalities including polyostotic fibrous dysplasia, pigmented skin lesions (cafe-au-lait spots), and autonomous hyperfunction of various endocrine organs including gonads, anterior pituitary, thyroid, and adrenal cortex. The endocrine abnormalities lead to precocious puberty, gigantism/acromegaly, hyperthyroidism, and hypercortisolism. The cause of this sporadic disorder has been enigmatic, but speculations have centered on a defect in signal transduction leading to endocrine hyperfunction. The distribution of skin lesions has also suggested the possibility of a somatic mutation acquired early in embryogenesis and affecting only a subset of cells (mosaicism). Since a G protein mutation could plausibly explain the endocrine manifestations, we searched for and found mutations of the Gs-alpha gene that lead to constitutive activation of the Gs protein. These mutations were found in a mosaic distribution; notably, mutant gene was undetectable in normal-appearing portions of endocrine glands, but was present at heterozygous levels in neoplastic portions of endocrine tissue. Mutant Gs-alpha was also detected in dysplastic bone lesions, both in the polyostotic, "classical" form of MAS and in a "form fruste" of the disease, monostotic fibrous dysplasia. Occurrence of mutant Gs-alpha in organs such as heart and liver suggest a possible role in "non-classical" manifestations, including sudden death. Our studies suggest that MAS is caused by a somatic mutation in the Gs-alpha gene occurring early in development and found in a mosaic distribution.More focal manifestations of the disease such as monostotic fibrous dysplasia may be caused by somatic mutation of the Gs-alpha gene occuring later in development.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-83X

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43304-01 MDB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Guanine nucleotide binding protein beta-gamma dimers: structure and function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William Simonds, M.D.,	Senior Clinical Investigator	MDB, NIDDK
Others:	Chunghee Lee, Ph.D.	Visiting Associate	MDB, NIDDK
	Shiying Zhang, Ph.D.	Visiting Fellow(since 1/94)	MDB, NIDDK

COOPERATING UNITS (if any)

Husseini Manji, M.D., ETB, NIMH
J. Silvio Gutkind, Ph.D. LCDO, NIDR
R. Collins, Ph.D., MDB, NIDDK

LAB/BRANCH Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS 2.75	PROFESSIONAL: 2.75	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The guanine-nucleotide binding regulatory proteins (G-proteins) are α1β1γ1 heterotrimers which function as transmembrane signal transducers by coupling receptors for extracellular stimuli to intracellular effectors (enzymes, ion channels). G-proteins constitute a diverse family distinguished by specific receptor and effector interactions which in turn are determined by the structure of the three constituent subunits. The α subunit binds guanine nucleotides and has a well established role in effector modulation. The β and γ subunits are tightly associated as a βγ complex, comprising a single functional entity which, like the α subunit, is absolutely required for G-protein interaction with receptor. An effector modulatory role for the βγ complex is becoming increasingly apparent in several systems. The present research emphasizes the role of the βγ complex in G-protein-mediated signal transduction. We have used subunit specific peptide antibodies to probe regions of the βγ complex important for functional interaction with the α subunit and to monitor expression of recombinant subunits. Site-directed mutagenesis has been used to study the assembly, processing and effector function of the βγ complex in both transient and stable transfected cell systems. These studies may elucidate the contribution of the βγ subunit complex to the receptor and effector selectivity characteristic of G-proteins and to the adaptive responses pursuant to agonist stimulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 43305-01 MDB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on nephrogenic diabetes insipidus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Spiegel, M.D.	Chief, MDB, NIDDK
Others:	J. Merendino, M.D.	Clinical Associate, MDB, NIDDK
	D. Wenkert, M.D.	Clinical Associate, MDB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION NIDDK, NIH Bethesda, MD

TOTAL STAFF YEARS 0.75	PROFESSIONAL: 0.5	OTHER: 0.25

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Nephrogenic diabetes insipidus (NDI) is an inherited X-linked disorder in which affected subjects are resistant to the actions of vasopressin (AVP) on renal medullary cells responsible for water concentration. Clinical manifestations include severe polydipsia and polyuria, and resultant severe dehydration can lead to cerebral swelling and death. Treatment with a potent AVP analog (DDAVP), useful in other forms of DI, is ineffective in NDI because of end-organ resistance to the hormone. The renal actions of AVP are mediated through a V2 type receptor linked via the Gs protein to stimulation of the 2nd messenger cAMP. In theory, the inherited gene defect could be located anywhere along the signal transduction path, but indirect evidence suggested a likely receptor defect. The recent cloning of a human V2 receptor permitted chromosomal localization studies which showed that the receptor is localized to Xq28, the site of the gene defect as determined by family linkage studies. This strongly suggested but did not prove that a receptor gene mutation is the underlying defect in NDI. We have obtained genomic DNA samples on multiple families with NDI, and in eight families thus far have identified mutations predicted to disrupt formation of a normal V2 receptor. These findings have important implications for our understanding of the pathogenesis of NDI and of normal V2 receptor structure and function, for identification of affected subjects and carriers, and eventually for gene therapy of the disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47001-12

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Insulin-like Growth Factor-1 Receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.'s:	D. LeRoith	Med Officer (Research)
	C.T. Roberts, Jr.	Research Biol.
Others:	V. Blakesley	Clinical Associate
	B. Stannard	Biologist
	H. Werner	Visiting Associate
	D. Beitner-Johnson	PRAT Fellow
	C. Hernandez-Sanchez	Visiting Fellow
	C. Sindler	IRTA

COOPERATING UNITS (if any)

R. Vaserga	Jefferson University (Philadelphia)
Y. Zick	Weizmann Institute (Rehovot, Isreal)
H. Kato	Utsunomiya University (Japan)

LAB/BRANCH

Diabetes Branch

SECTION

Section of Molecular and Cellular Physiology

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.0	7.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Insulin-like growth factor-I receptor (IGF-IR) is a tyrosine kinase transmembrane glycoprotein. The insulin-like growth factor-I receptor promoter is very GC rich as is the long 5' untranslated region (UTR) and there are a number of consensus sites for Wilm's Tumor suppressor gene product (WT1) and Sp1 binding including a newl described "CT" element. Co-transfection of the insulin-like growht factor-I receptor promoter driving a luciferase reporter gene with vectors containing Wilm's Tumor suppressor gene of Sp1 cDNAs demonstrated that Wilm's Tumor inhibited, wheras Sp1 enhanced, promoter activity. In addition cells expressing Wilm's Tumor had inherently lower insulin-like growth factor-I receptor promoter activity and lower insulin-like growht factor-I receptor mRA levels. These results tend to explain the higher levels of insulin-like growth factor-I receptors in Wilm's tumor compared to normal adjacent kidney tissue.

Mutations of the insulin-like growth factor-I receptor have shed light on the structure and function aspects of this tyrosine kinase receptor. Mutation of ATP-binding site and the triple tyrosine cluster in the kinase domain abrogates all functional aspects. Substitution of only one tyrosine (1131) of this cluster, while decreasing subunit autophoshorlation did not significantly alter biological actions. Truncation of the C-terminal 108 amino acids of the insulin-like growht factor-I receptor caused a marked diminution of autophosphorylation, but minimal changes in IRS-1 hosphorylation and no effect on mitogenic activity 100 amino acids of the insulin and insulin-like growht factor-Ireceptors were swithche, had a greater effect on the function of the insulin receptor. These studies suggest that despite marked reductions in subunit autophosphorylation, if IRS-1 is phosphorylated fundamental functions are retained. Also, it appears that the insulin-receptor and insulin-like growht factor-I receptor C terminii are inherently different and may explain the different physiological functions for each receptor seen in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK47005-22 DB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies of insulin receptors in circulating cells in man

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute

P.I.	P. Gorden	Director	NIDDK
Others:	C. Hendricks	Biol. Lab. Tech.	DB, NIDDK
	P. Roach	Senior Staff Fellow	DB, NIDDK
	P. Formisano	Special Volunteer	DB, NIDDK
	S.I. Taylor	Chief	DB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The present work continues prior investigation of insulin receptors on circulating cells in patients with insulin resistance and diabetes mellitus. Insulin receptors are evaluated for their ability to bind insulin and to act as tyrosine-specific protein kinases. We have specifically studied two individual patients: one with a Type A form of insulin resistance that binds insulin normally but has reduced tyrosine-kinase activity, and the second, a patient with a Rabson-Mendenhall Syndrome, who has a severe defect in insulin binding.

In addition, we have continued to study patients with autoantibodies to the insulin receptor associated with insulin resistance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47007-18 DB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antibodies to Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	S. Taylor	Chief, DB/NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Diabetes Branch

SECTION

Receptors & Hormone Action

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47009-07 DB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Positron Emission Tomography/NMR Spectroscopy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.	R. Eastman	Director	DDEMD,NIDDK
Others:	S. Taylor	Chief	DB,NIDDK
	D. LeRoith	Section Chief	DB,NIDDK
	C. Cochran	RN	CC
	B. Koller	Senior staff Fellow	DB, NIDDK
	M. Skarulis	Clinical Investigator	NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Diabetes Branch

SECTION

Receptors and Hormone Action

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland

TOTAL STAFF YEARS: 2	PROFESSIONAL: 2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47018-16 DB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Insulin-Like Growth Factors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.'s:	D. LeRoith	Section Chief	E. Hernandez	Guest Researcher
	C.T. Roberts, Jr.	Research Biol.	D. Landau	Guest Researcher
Others:	S. Neuenschwander	Visiting Fellow		
	A. Koval	Visiting Fellow		
	Z. Shen-Orr	Guest Researcher		

COOPERATING UNITS (if any)

J. Fontana (Univ. Maryland, Baltimore, MD)
V. Kavsan Kiev, Ukraine
A. Jaffa (Univ. S. Carolina, Charleston, SC)

LAB/BRANCH

Diabetes Branch

SECTION

Section of Molecular and Cellular Physiology

INSTITUTE AND LOCATION

National Institute of Diabetes, Digestive and Kidney Diseases, NIH 10/8D48

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Summary The insulin-like growth factor family comprises two peptides, (insulin-like growth factor-I and insulin-like growth factor-II), six binding proteins, (insulin-like growth factor binding protein 1-6) and two receptors. They are widely expressed and play an essential role in the growth and development of all tissues, as well as in the normal functioning of differentiated tissues. The gene for insulin-like growth factor-I is very complex and its expression is regulated at multiple levels. Its action may be modulated by the insulin-like growth factor binding proteins either by enhancement or inhibition.

Insulin-like growth factor-I and the insulin-like growth factor binding proteins are important in development of the ovary and in particular in granulosa cell maturation and steroidogenesis. Cancer cells express the insulin-like growth factor binding proteins in different combinations and in response to different factors. These insulin-like growth factor binding proteins can modulate the effect of insulin-like growth factor-I on cancer cell proliferation. Diabetic kidney disease is associated with hemodynamic changes which are most probably induced by insulin-like growth factor-I which accumulates in the kidney due to increased kidney insulin-like growth factor binding protein-1 expression. Finally, the Insulin-like growth factor system apparently plays a significant role in splenic growth and development and therefore may be an important factor in immune responsiveness.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47019 18 DB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Morphologic studies of ligand binding to cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I. Phillip Gorden Director NIDDK

COOPERATING UNITS (if any)

Institute of Histology and Embryology, University of Geneva School of Medicine, Geneva, Switzerland. (J.L. Carpentier, L. Orci) - Foreign

LAB/BRANCH

Diabetes Branch

SECTION

Clinical and Cellular Biology Section

INSTITUTE AND LOCATION

NIDDK, NIH Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This work represents a 18-year collaboration between the Diabetes Branch and the Institute of Histology and Embryology at the University of Geneva. The initial observations demonstrated that polypeptide hormones are taken up by the cell through a process of receptor-mediated endocytosis similar to other biologically important ligands that bind to cell surface receptors. Several lines of work have been followed in the present project. (a) We have studied the entire process of endocytosis in the diabetic rat, including both receptor-mediated and fluid-phase endocytosis; and (b) We have studied the localization of newly synthesized insulin receptors under conditions of site specific mutagenesis of glycosylation sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47022 - 15 DB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular genetics of insulin resistance and noninsulin-dependent diabetes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.	S.I. Taylor	Chief	DB, NIDDK				
Others:	M. Lesniak	Chemist	DB, NIDDK	D. Accili	Vis. Sci	DB, NIDDK	Y. Zick, DB
	M. Quon	Clin. Assoc.	DB, NIDDK	R. L-Toledano	Vis. Assoc.	DB,NIDDK	G. DiCola, DB
	E. Wertheimer	Vis. Fel.	DB, NIDDK	M. Sierra	Biologist	DB, NIDDK	
	C. Haft	IRTA	DB, NIDDK	E. Koller	Staff Fell.	DB, NIDDK	
	Y. Imai	Spec. Vol.	DB, NIDDK	J. Hone	Clin.Assoc.	DB, NIDDK	
	Y. Suzuki	Vis. Assoc.	DB, NIDDK	L. Sorbara	SSF	DB, NIDDK	
	M. Cool	Biologist	DB, NIDDK	M. Taouis	Vis. Fel.	DB, NIDDK	

COOPERATING UNITS (If any)

Axel Ullrich, Munich, Germany; A. Cama, Chieti, Italy; T. Kadowaki, Tokyo, Japan; HW Rödiger, and M. Dreyer, Hamburg, Germany; F. Barbetti, Rome, Italy; LI Al-Gazali, United Arab Emirates; L. Sinclair, London, GB; D. Moller, Boston, MA; J. Roth, Baltimore, MD; M. Muggeo, Verona, Italy; Y. Litvin, New York; A. Dunaif, Hershey, PA;

LAB/BRANCH

Diabetes Branch

SECTION

Receptors and Hormone Action

INSTITUTE AND LOCATION

NIDDK

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
10.5	8.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Insulin resistance contributes to the pathogenesis of several disease states including obesity and noninsulin-dependent diabetes mellitus (NIDDM). We have investigated the nature of the genetic factors that predispose to the development of insulin resistance. Mutations have been identified in the insulin receptor gene in patients with several genetic syndromes associated with insulin resistance. Some mutations cause insulin resistance by decreasing the number of receptors on the cell surface by one of several mechanisms: impairing receptor biosynthesis, impairing the transport of receptors to the plasma membrane, or accelerating the rate of receptor degradation. Other mutations cause insulin resistance by decreasing the affinity of insulin binding, or impairing receptor tyrosine kinase activity. To investigate the significance of heterozygosity for a mutation in the insulin receptor gene, we have investigated the effect of mutations in hybrid receptors formed by dimerization of mutant with wild type half-receptor. In the case of the Leu-323 mutation (a mutation in the extracellular domain that inhibits insulin binding), the hybrid receptor bound insulin with normal affinity. This explains the observation that the Leu-323 mutation causes insulin resistance in a recessive fashion. In contrast, mutations in the intracellular domain (e.g., Ile-1153 mutation) generally inhibit receptor tyrosine kinase activity, and cause insulin resistance in a dominant fashion. However, Ile-1153 mutant receptors do not appear to dimerize with wild type receptors when both cDNA's are expressed in the same cells. The dominant effect of the Ile-1153 mutation may be explained by competition for binding of downstream effector molecules. However, most patients with NIDDM appear not to have mutations in the insulin receptor gene. Thus, we have screened for the presence of mutations in the gene encoding insulin receptor substrate-1 (IRS-1). Several variant sequences have been identified in the IRS-1 gene, and the prevalence of these variant sequences appears to be increased in patients with NIDDM. Studies are underway to determine whether these variant sequences impair the function of IRS-1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47024-15 DB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthetic labeling of the insulin receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.	P. Gorden	Director	NIDDK
Others:	S.I. Taylor	Chief	DB/NIDDK

COOPERATING UNITS (If any)

J-L Carpentier, Geneva, Switzerland
L. Beitz, Howard Hughes, Bethesda; E. Collier, CIB/NIAID

LAB/BRANCH

Diabetes Branch

SECTION

Clinical and Cellular Biology Section

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One of the post translational modifications, N-linked glycosylation, of the insulin receptor has been studied in cultured cells. Using an experimental approach based on mutagenesis of the insulin receptor complimentary DNA (cDNA) at specific sites of potential modification, the mutated cDNA molecules are stably transfected into cultured cells, and then the structure and function of these mutant receptors can be studied. Processing of these mutants was investigated by biosynthetic labeling. Cells unable to glycosylate their receptors in the first four potential glycosylation sites are abnormally processed. These receptors do not appear on the cell surface and remain in proreceptor form in the endoplasmic reticulum. Mutants for each of these sites, individually and in combination, also are abnormally processed. However, the severity of the defect is dependent on the site of the mutation and the number of sites mutated. The first or the second site can be mutated with very little effect on the concentration of receptor processed. However, combination of mutations in both of these sites has a dramatic effect on the processing of the receptor, whereas the combination of mutations in the third and fourth sites has less of an effect on the processing of the receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47026 - 10 DB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Insulin receptors: structure, function, and role in insulin action

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute

P.I.	S.I. Taylor	Chief	DB, NIDDK		
Others	R. Levy-Toledano	Visiting Associate	DB, NIDDK	S. Cushman	Res. Chem., EDMNS,DB
	D. Accili	Visiting Scientist	DB, NIDDK	MJ Zarnowski	Biol., EDMNS,DB
	N. Hindman	Special Volunteer	DB, NIDDK	M. Guerre-Millo	Spev. Vol.,EDMNS,DB
	S. Najjar	IRTA	DB, NIDDK		
	M. Quon	Clinical Associate	DB, NIDDK		
	A. Butte	Guest Researcher	DB, NIDDK		
	Y. Suzuki	Visiting Associate	DB, NIDDK		

COOPERATING UNITS (if any)

R.D. Klausner, CBMB, CH.

LAB/BRANCH

Diabetes Branch

SECTION

Receptors and Hormone Action

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.75	2.75	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the first step in insulin action, insulin activates the insulin receptor tyrosine kinase. This leads to phosphorylation of tyrosine residues in multiple intracellular proteins. We have investigated this pathway in isolated rat adipocytes, a physiologically important target cell for insulin action. When recombinant epitope tagged GLUT4 glucose transporters are expressed in adipocytes, insulin stimulates the recruitment of these glucose transporters to the cell surface. When human insulin receptors are co-expressed in rat adipocytes, this leads to a marked increase in the number of epitope tagged glucose transporters on the cell surface. In contrast, Ile-1153 mutant insulin receptors are deficient in tyrosine kinase activity, and do not affect the number of epitope-tagged glucose transporters when co-expressed in rat adipocytes. Thus, tyrosine kinase activity is required for the ability of insulin receptors to mediate the effect of insulin to stimulate glucose transport in rat adipocytes. Thus, it is of interest to determine which intracellular proteins function downstream from the receptor tyrosine kinase in the pathway that leads to translocation of GLUT4 molecules to the cell surface. Insulin receptor substrate-1 (IRS-1) is among the best characterized substrates for phosphorylation by the insulin receptor. When IRS-1 levels were decreased by expression of an anti-IRS-1 ribozyme (designed to cleave rat IRS-1 mRNA), this impaired the ability of insulin to stimulate the translocation of epitope-tagged GLUT4 to the cell surface in isolated rat adipocytes. Furthermore, expression of human IRS-1 in rat adipocytes increased the number of epitope-tagged GLUT 4 molecules on the cell surface These observations suggest that IRS-1 mediates (at least in part) the effect of insulin to stimulate glucose transport. IRS-1 undergoes multi-site phosphorylation, thereby activating multiple SH2-domain-containing proteins that function in the insulin action pathway. Because expression of a dominant negative mutant of phosphatidyl inositol 3-kinase (PI-3-K) inhibits insulin action in rat adipocytes, this suggests that PI-3-K functions downstream from IRS-1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 47027 - 09 DB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Use of Somatostatin Analog (SMS 201 - 995) in hormone secreting tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.	R.C. Eastman	Director	DDEMD, NIDDK
OTHERS:	C. M. Hendricks	Bio Lab Tech.	DB, NIDDK
	P. Gorden	Director	NIDDK
	P. Roach	Clinical Assoc.	DB, NIDDK

COOPERATING UNITS (If any)

B. Weintraub	Chief, MCNEB, NIDDK

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This is a project to study the long-term effects of the treatment of acromegaly with the long-acting somatostatin analog, octreotide. Patients are treated in an unblinded fashion with octreotide as long as it is medically necessary. Treatment and prevention of gallbladder sludge and gallstones, common side effects of long-term octreotide therapy, is being attempted with ursodeoxycholate, a bile salt which has been used to dissolve gallstones in the general population. Patients who are candidates for treatment with octreotide, and who have normal gallbladders as determined by ultrasonography, are randomized to receive either ursodeoxycholate or placebo in order to study whether ursodeoxycholate is effective in preventing octreotide-induced gallbladder sludge or stones are treated in an unblinded fashion with ursodeoxycholate in an attempt to dissolve the sludge or stones. Fifteen patients have been randomized to masked treatment with ursodeoxycholate, and nine patients have been treated unmasked for sludge or stones. To date, pre-existing gallstones have dissolved in 3 of 5 patients and pre-existing sludge has disappeared in 2 of 4 patients during ongoing therapy with octreotide. We are continuing to enroll patients in the randomized prospective trial, as well as to treat with ursodeoxycholate all patients who develop octreotide-induced gallbladder abnormalities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47028 - 05 DB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of genes encoding proteins that mediate insulin action

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.	S.I. Taylor	Chief	DB, NIDDK
Others:	H. Chen	Special Volunteer	DB, NIDDK
	Sonia Najjar	IRTA	DB, NIDDK
	D. Accili	Visiting Scientist	DB, NIDDK
	N. Philippe	Biological Aide	DB, NIDDK

COOPERATING UNITS (if any)

Catherine McKeon, DDEM, NIDDK; Guc Ooi, GD, MCEB, NIDDK; Gillian Walker, Australia.

LAB/BRANCH

Diabetes Branch

SECTION

Receptor and Hormone Action Section

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Regulation of the number of insulin receptors on the cell surface plays a critical role in determining insulin sensitivity. In order to study the mechanism of transcriptional regulation of the insulin receptor gene, we have cloned the 5'-end of the human and mouse insulin receptor genes. Recently, we have characterized trans-acting factors that bind to a 26 base pair enhancer located 503-528 base pairs upstream from the translation start site. This enhancer accounts for a two- to threefold increase in the level of gene expression. Using electrophoretic mobility shift analysis, we have demonstrated at least two proteins that bind to overlapping sequences in the enhancer. Although the enhancer sequence does not contain a perfect match to the consensus Sp1 binding motif, there appears to be a relatively low affinity binding site for Sp1. In addition, a second protein binds to the enhancer sequence. Although the identity of this second DNA binding protein has not been determined, it appears to be a novel factor. While the insulin receptor mediates the first step in insulin action, other proteins function downstream in the insulin action pathway. We have initiated studies into the regulation of the gene encoding pp120/HA4, a membrane glycoprotein in rat liver that undergoes insulin-stimulated tyrosine phosphorylation. Consistent with the tissue-specific expression in liver, the pp120/HA4 gene has a TATA box containing promoter. In addition, the 5'-flanking region of the gene contains putative binding sites for several factors including a glucocorticoid response element and an insulin response element. We have initiated studies to investigate the regulation of expression vectors in which the promoter plus 5'-flanking region of the pp120/HA4 gene drive the expression of the luciferase gene. Using this approach, we have begun to map cis-acting elements responsible for the ability of insulin and dexamethasone to increase gene expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 47029 - 03 DB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mathematical modeling of glucose metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.	M. Quon	Clinical Associate	DB/NIDDK
Others:	R. Eastman	Director	DDEMD/NIDDK
	S.I. Taylor	Chief	DB/NIDDK

COOPERATING UNITS (If any)

LAB/BRANCH
Diabetes Branch

SECTION
Receptors & Hormone Action

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Insulin resistance occurs in a variety of pathological states including obesity, non-insulin dependent diabetes mellitus (NIDDM) and hypertension. Decreased glucose effectiveness has been reported in NIDDM. Since insulin resistance and impaired glucose effectiveness may play a role in the pathogenesis of NIDDM, quantitative assessment of these properties is of interest. Recently, a minimal model approach involving mathematical modeling and computer simulation has been used to estimate both insulin sensitivity and glucose effectiveness from the results of a single frequently sample intravenous glucose tolerance test (FSIVGTT). The equations of the minimal model describe changes in plasma glucose concentration as functions of insulin and glucose concentrations. A computer program identifies model parameters that generate a best fit to insulin and glucose data obtained during the FSIVGTT. Thus, the minimal model is able to estimate the relative contributions of insulin and glucose to glucose tolerance. We have used the minimal model to generate specific predictions of both insulin-dependent and insulin-independent glucose metabolism under a variety of conditions. We performed experiments in subjects with insulin dependent diabetes mellitus and in normal subjects to test these predictions. By comparing model predictions with experimental results, we were able to demonstrate that the minimal model underestimates the contributions of insulin and overestimates the contribution of glucose to glucose metabolism. Studies are presently underway to confirm these results and to understand the origin of the discordance between minimal model predictions and experimental results. The data that we collect may allow us to formulate a more accurate and useful mathematical model.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47030 - 03 DB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Insulin receptor-related receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.	S.I. Taylor	Chief	DB, NIDDK
Others:	H.Y. Jui	Special Volunteer	DB, NIDDK
	Y. Suzuki	Visiting Associate	DB, NIDDK
	D. Accili	Visiting Scientist	DB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Diabetes Branch

SECTION

Receptors & Hormone Action

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The insulin receptor is encoded by a single copy gene located on chromosome 19 in the human. In addition, two other homologous genes have been identified. One gene encodes the type 1 insulin-like growth factor (IGF) receptor. The third gene in the family encodes an orphan receptor tyrosine kinase named the insulin receptor-related receptor (IRR). We have cloned the cDNA molecules encoding human and mouse IRR. Generally, the predicted structure of IRR is similar to those of the receptors for insulin and IGF-1. In the case of human IRR, we detected a splicing variant in which 24 nucleotides from the 3'-end of intron 13 are included between the sequences of exons 13 and 14. Two observations suggest that this alternatively spliced form may not be physiologically important. First, this variant species of IRR mRNA represents <10% of IRR mRNA in adult human kidney. Second, this splicing variant is not evolutionarily conserved; although there is a potential alternate splice acceptor site 23 nucleotides from the end of intron 13 in mouse IRR, this alternate splicing pattern would be predicted to shift the reading frame. Several variant splicing patterns were observed in the region of the junction between exons 1 and 2 of mouse IRR. None of these variant species of mouse IRR mRNA conserved the open reading frame. Inasmuch as they are not predicted to encode functional IRR molecules, it is unlikely that they are physiologically significant. When IRR cDNA is expressed by transfection in NIH-3T3 cells, the IRR precursor undergoes post-translational processing steps including N-linked glycosylation and proteolytic cleavage into distinct alpha and beta subunits. When cells expressing recombinant IRR are incubated with vanadate (an inhibitor of phosphotyrosine phosphatases), this leads to an increase in the phosphotyrosine content of IRR. However, the phosphotyrosine content of IRR is not increased by incubating the cells with insulin, IGF-1, or proinsulin. Further investigations are underway to attempt to identify the natural ligand for IRR, and to determine the physiological role of IRR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47031-03 DB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.	M. Reitman	Senior Staff Fellow	DB, NIDDK
Others:	Oksana Gavrilova	Visiting Fellow	DB, NIDDK
	Yufang He	IRTA	DB, NIDDK
	Mark Mason	IRTA	DB, NIDDK

COOPERATING UNITS (if any)

Heiner Westphal and Eric Lee, LMG, NICHD; Joseph Grasso, University of Connecticut Health Center; Thomas L. Vandergon, LMB, NIDDK.

LAB/BRANCH

Diabetes Branch

SECTION

Molecuar Biology and Gene Regulation

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our goal is to understand how multi-gene clusters are organized and regulated. Specifically, 1) how does the physical organization influence expression of the genes, 2) what are the mechanisms that operate to 'open' chromatin, and 3) how is a particular gene within an open cluster chosen for expression? The model evolving from studies of the human ß-globin cluster is that control elements upstream of the genes are essential for locus activation (chromatin opening). These upstream elements also increase the expression of the nearest available genes, with availability chiefly determined by the promoter. This model is supported by the genotype phenotype correlations observed in patients with deletional ß-thalassemia and hereditary persistence of fetal hemoglobin.

To examine the contribution of the upstream hypersensitive sites and ß^A 3' enhancer in the regulation of each of the globin genes in the intact cluster, we introduced the entire chicken ß-globin cluster into mice. All four transgenes were expressed with the correct developmental pattern, suggesting that the stage-specific erythroid transcriptional milieus existed before the divergence of aves and mammals. Deletion of either the upstream hypersensitive sites or of the ß^A 3' enhancer caused reduced expression of all four globin transgenes. Thus, all of the genes are under the control of both the upstream hypersensitive sites and the enhancer and the influence of the control elements can extend beyond the nearest active gene.

Each of the four hypersensitive sites found upstream of the cluster was tested for enhancer activity. HS2 and HS3 showed significant enhancer activity and HS2 was studied in detail. Four binding sites for the GATA-1 transcription factor were found and accounted for the enhancer activity.

We have also sequenced the cluster. This revealed four gene conversions and allowed deduction of the order of gene duplication. CR1 repetitive elements accounted for 16% of the cluster, a surprisingly large fraction, suggesting that the cluster is a hot spot for CR1 insertion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 48001 02 DB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Insulin Cell Interaction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I:	Ian A. Simpson	Visiting Scientist	DB/NIDDK
Others:	Samuel W. Cushman	Chief, EDMNS	DB/NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Diabetes Branch

SECTION
Experimental Diabetes, Metabolism, and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH Bethesda, Md. 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 48002-03 DB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Insulin's Regulation of Glucose Transport

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)*
PI: Samuel W. Cushman Chief, EDMNS DB/NIDDK
Others: I.A. Simpson, Visiting Scientist, DB/NIDDK; M. Millo, Special Volunteer, DB/NIDDK; K.I. Timmers, Special Volunteer, DB/NIDDK; C.M. Wilson, IRTA, DB/NIDDK; M. Omatsu-Kanbe, Visiting Fellow, DB/NIDDK; T. Ploug, Special Volunteer, DB/NIDDK; J.T. Brozinick, IRTA, DB/NIDDK; C.S. Hinck, Special Volunteer, DB/NIDDK; T.H. Reynolds, preIRTA, DB/NIDDK; D.G. Patel, Special Volunteer, DB/NIDDK; D.R. Yver, Chemist, DB/NIDDK; M.J. Zarnowski, Biologist, DB/NIDDK; M.J. Quon, Clinical Associate, DB/NIDDK.

COOPERATING UNITS *(if any)*
Yokohama University School of Medicine, Shizuoka General Hospital, The University of Bath, Receptron, Inc., East Carolina University School of Medicine, Emory University School of Medicine, Stanford University School of Medicine, Palo Alto Medical Foundation, Baylor College of Medicine, Harvard Medical School, Tufts-New England Medical Center, University of Maryland.

LAB/BRANCH
Diabetes Branch

SECTION
Experimental Diabetes, Metabolism, and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
10.2	10.2	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A study of the subcellular trafficking of tracer-tagged GLUT4 in rat adipose cells shows that 1) insulin does not alter GLUT4 endocytosis but instead increases the rate of exocytosis, 2) all of the cells' GLUT4 is involved in the recycling process, and 3) plasma membrane intermediate states may be present in the GLUT4 trafficking pathways. A mathematical analysis has been carried out which shows that this kinetic behavior is consistent with a 3-state model, with two intracellular compartments, and the presence of occluded states in the plasma membrane. Peptides from the alpha 1 domain of the major histocompatibility complex class I antigen (MHC class I) enhance cellular glucose uptake above that of maximal insulin stimulation, prolong the effect of insulin, and inhibit insulin receptor internalization in rat adipose cells, perhaps through regulation of the internalization process of cell surface integral membrane proteins. We also have observed that K^+ depletion inhibits GLUT4 internalization strongly suggesting the involvement of a coated pit mechanism. Staurosporine, a widely used protein kinase C inhibitor, completely inhibits both PMA- and insulin-stimulate glucose transport activity in isolated rat adipocytes, possibly at a step that regulates the specific translocation of the glucose transporters to the plasma membrane. We have used a photoaffinity label to quantify cell surface GLUT4 glucose transporters in isolated rat soleus muscles; the insulin-stimulated increase in glucose transport activity appears to be accounted for by an increase in surface-accessible GLUT4 content. Autoradiography of photolabeled soleus muscles supports the concept that glucose transport may occur predominately across the T-tubule membrane. We have used the same photoaffinity label to quantitate the glucose transporters on the surface of BC3H-1 cells. This technique demonstrates that glucose prefeeding 1) decreases the total number of GLUT1 and 2) redistributes the majority of the remaining transporters to an intracellular site where they can now be translocated to the cell surface in response to insulin and PMA. We have observed that LDL receptors localized in intracellular compartments of isolated rat adipose cells seem to be functionally regulated since insulin acutely diminishes the number of receptors by accelerating their rate of degradation. We tested the hypothesis that sulfonylureas promote glucose uptake into 3T3-L1 cells or isolated rat adipocytes through specific high affinity receptors. We conclude that the anti-diabetogenic effects of sulfonylureas are not mediated by a direct action of sulfonylureas to increase glucose uptake into adipose tissue. We have observed that chronic consumption of a diet containing trans-fatty acids can influence lipid metabolism and result in decreased fat accumulation in murine adipose tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 48003-03 DB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Alterations in Insulin's Action in Insulin-Dependent Diabetes Melliuts

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Sameul W. Cushman Chief, EDMNS DB/NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Diabetes Branch

SECTION
Experimental Diabetes, Metabolism, and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20893

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 48005-03 DB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Counterregulation of Insulin's Action by Catecholamines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)*

PI:	Ian A. Simpson, Ph.D.	Visiting Scientist	EDMNS/DB/NIDDK
Others:	Samuel W. Cushman, Ph.D.	Chief	EDMNS/DB/NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Diabetes Branch

SECTION
Experimental Diabetes, Metabolism, and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inactive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-DK 48007-02 DB

PERIOD COVERED
October 1, 1993 to September 31, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Glucose Transport in Mammalian Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)*

Ian A. Simpson, Ph.D.	Visiting Scientist	EDMNS/DB/NIDDK
Frances Maher, Ph.D.	Visiting Fellow	EDMNS/DB/NIDDK
Lynn R. Sorbara, Ph.D.	Staff Fellow	EDMNS/DB/NIDDK
Theresa M. Davies-Hill	Biologist	EDMNS/DB/NIDDK
Mellonie A. Boyd	Special Volunteer	EDMNS/DB/NIDDK
Kang Li, M.D., Ph.D.	Special Volunteer	EDMNS/DB/NIDDK

COOPERATING UNITS *(if any)*
Dr. P.Davies/Albert Einstein College of Medicine, Dr. P.Lysko/SmithKline Beecham, Dr. K.Seamon/CBER/DBB/FDA, Dr. C.Smith/NIMH/NIH, Dr. J.Latterra/Johns Hopkins, Dr. G.Dichiria/NINDS/NIH, Dr. R.Sherwin/Yale U., Dr. M. Birnbaum/Harvard, Dr. M.Levine/NIDDK/NIH, Dr. C.Burant, U. Chicago, Dr. S.Vannucci/Hershey Medical Center

LAB/BRANCH
Diabetes Branch

SECTION
Experimental Diabetes, Metabolism, and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bldg. 10, Rm. 5N102, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.4	4.0	1.4

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The brain is dependent on glucose to fuel its energy demands. Due to the heterogeneous nature of brain cells, primary neuronal culture systems are useful for the study of neural glucose transport. Two glucose transporter isoforms GLUT1 and GLUT3 have been detected in cerebellar granule cells in culture. A these cells differentiate, their glucose transport activity is enhanced with a corresponding increase in the expression of both GLUT1 and GLUT3. Increases in mRNA and protein provide no insight into which is functionally the more important transporter. We have determined the relative concentrations of each transporter isoform using three different labelling techniques. Biosynthetic labelling of both transporters with [^{35}S]methionine and cysteine revealed GLUT3 to be 6-10-fold more abundant than GLUT1. A similar ratio of GLUT3:GLUT1 was observed using an impermeant bis-mannose photolabel ([^{3}H]ATB-BMPA). Labelling the transporters with the permeant photolabel [^{125}I] AIPP-forskolin revealed 5-8 fold more GLUT3 than GLUT1, whereas the ratio of GLUT3:GLUT1 was only 1:1 in rat brain membranes. Quantitatively, GLUT3 is the predominant transporter in these neurons *in vitro* and if this pertains to neurons *in vivo*, it seems likely that GLUT1 is the primary glial glucose transporter. Our recent studies support a direct relationship between GLUT3 expression and rates of glucose utilization. Chronic depolarizing stimuli *in vitro*, e.g. high K^+ and the glutamate agonist N-methyl-D-aspartate (NMDA), which increase energy demand due to repolarization, result in enhanced glucose transport activity and expression of GLUT1 and GLUT3 in granule cells. We have observed a similar relationship in our *in vivo* studies. Dehydration due to water deprivation and streptozotocin-induced diabetes both activate the hypothalamo-neurohypophyseal system for vasopressin secretion;dehydration has been shown to increase glucose utilization in the neurohypophysis (NH). Both conditions resulted in marked (50%) increase in GLUT3/mg protein in the NH. However, whereas dehydration caused an increase in GLUT1 expression, diabetes induced a reduction in GLUT1. The increases in GLUT3 expression in these conditions represents the first demonstration of GLUT3 regulation in response to a common hypothalamic signal. The direct relationship between glucose utilization and GLUT3 also applies in the converse. Patients with Alzheimer's disease demonstrate significant reductions in cerebral glucose utilization, as determined by PET scanning, especially in parietal and temporal cortex. Analysis of brain tissue obtained from patients with Alzheimer's disease for GLUT1 and GLUT3 levels revealed reductions of both isoforms in cerebral cortex, with highly significant reductions in GLUT3 in parietal and temporal cortex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 52008-15 GBB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Gene Expression and Human Genetics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R.D. Camerini-Otero Chief GBB, NIDDK

Others:

E. Angov	IRTA Fellow	J. Yancey-Wrona	IRTA Fellow
L. Ferrin	Research Associate	G. Poy	Biologist
Hai Kinal	IRTA Fellow	Oleg Voloshin	Visiting Associate
Lijiang Wang	IRTA Fellow	Sergei Kozyavkin	Visiting Associate
Vlad Malkov	Visiting Fellow		

COOPERATING UNITS (if any)
Carol Camerini-Otero - Diabetes Branch, NIDDK
Victor Zhurkin and Robert Jernigan - Laboratory of Mathematical Biology, NCI
Asad Sheikh - Division of Intramural Research, NCHGR

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS: 10.00	PROFESSIONAL: 9.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In order to dissect the biochemical steps involved in genetic recombination we have chosen to focus on a key early step: strand exchange between homologous parental DNAs. *In vitro*, the product of this strand exchange reaction is a joint molecule composed a single-strand DNA joined to one end of a linear duplex DNA. We have established a new paradigm for this homologous pairing, in essence, that recombinases such as the *E. coli* RecA protein can hybridize a single strand of any sequence and an intact duplex. That is, the three strands form a novel DNA triplex (R-form DNA) in which the third strand may have any arbitrary sequence and must have a parallel orientation with respect to the phosphodiester backbone of the identical strand in the duplex. We have also been able to isolate synaptic complexes consisting of all the three strands and RecA. These structures have been studied in detail and will continue to be the basis of additional structural investigations. The kinetics of formation of these complexes have been used as a model for the homology search process in the obligatory recombination events during meiosis. The data shows that the search for homology is a very fast step that is not rate-limiting and that this is followed by a very slow step involving conformational changes of the protein and the DNA. Several of the rate constants in this reaction have been measured. Evaluating these rate constants has allowed us to delimit the possible structures involved at each step. In order to understand the mechanism and structures involved in greater detail we have determined that a 20 amino acid peptide from RecA has some of the activities of RecA. Efforts are now underway to determine the three-dimensional structure of this peptide when bound to DNA. The synaptic complexes have also been used to develop a method for the selective cleavage of human DNA (RecA-Assisted Restriction Endonuclease (RARE) cleavage). This method has been shown to be useful to map across "gaps" in physical maps and to excise the very ends of chromosomes, that is, telomeres and subtelomeric DNA. Most recently, we are using this technique to map across several gaps in the completed physical map of chromosome 21 and to map and excise centromeres from human chromosomes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 52011-09 GBB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Toxins and DNA Repair in Xenopus Oocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Eric Ackerman	Senior Staff Fellow	GBB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been transferred to Z01 DK 11500-01 ODIR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 52012-10 GBB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure-Function Relationships of Lysosomal Enzymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard L. Proia	Research Biologist	GBB, NIDDK
Others:	Debra Boles	IRTA	GBB, NIDDK
	Olivia N. Johnson	Summer IRTA	GBB, NIDDK
	Francine Norflus	Biologist	GBB, NIDDK
	Kazunori Sango	Visiting Fellow	GBB, NIDDK
	Mark Pennybacker	IRTA	GBB, NIDDK
	Shoji Yamanaka	Visiting Associate	GBB, NIDDK

COOPERATING UNITS (if any)

Jacqueline N. Crawley, NIMH, NIH; Christine A. Kozak, NIAID, NIH; Ruth Navon, Saphir Medical Center, Kfar-Sava, Israel Kinuko Suzuki, Department of Pathology, Univ. North Carolina at Chapel Hill; Heiner Westphal, NICHD, NIH

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

Biochemical Genetics Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
5.45	5.25	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mutations in any of three genes, *HEXA*, *HEXB* and *GM2A*, result in forms of the G_{M2} gangliosidoses, a group of severe neurodegenerative diseases. We are in the process of creating models of these genetic disorders through targeted gene disruption in the mouse.

HEXA - We have produced mice with biochemical and neuropathologic features of Tay-Sachs disease by disruption of the *Hexa* gene encoding the α subunit of β-hexosaminidase A. The mutant mice displayed less than 1% of normal β-hexosaminidase A (β-hex A) activity and accumulated G_{M2} ganglioside in brain in an age-dependent manner. The accumulated ganglioside was stored in neurons as membranous cytoplasmic bodies characteristically found in the neurons of Tay-Sachs disease patients.

HEXB - The *Hexb* gene encoding the β subunit of β-hex A has been disrupted in embryonic stem cells and chimeric mice have been derived. When the homologous gene is defective in humans, Sandhoff disease results.

GM2A - The G_{M2} activator protein forms a substrate-complex with G_{M2} ganglioside which enables degradation of the ganglioside by β-hex A. Mutations in the human G_{M2} activator protein gene result in the G_{M2} gangliosidosis AB variant. We have isolated and sequenced a full-length mouse G_{M2} activator protein cDNA. The *Gm2a* gene was mapped to a region on mouse Chromosome 11 that is homologous with a segment of human chromosome 5 containing the orthologous human gene. In addition, a *Gm2a* related sequence (*Gm2a-rs1*) was mapped to mouse Chromosome 5.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 52015-05 GBB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Studies of Protein-DNA Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peggy Hsieh	Expert	GBB, NIDDK
Others:	Igor G. Panyutin	Visiting Associate	GBB, NIDDK
	Indranil Biswas	Visiting Fellow	GBB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
2.1	2.0	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are characterizing a key step in genetic recombination, DNA branch migration. Pairing of two homologous DNA duplexes and strand exchange carried out by recombination proteins like *E. coli* RecA lead to the formation of a central intermediate in recombination, the Holliday junction. The Holliday junction, corresponding to the exchange point between two homologous DNAs, can migrate by the step-wise exchange of hydrogen bonds between identical strands of the two DNA duplexes. This process is known as branch migration, and it dictates the extent of transfer of genetic information between two homologous chromosomes. Understanding the mechanics of branch migration is critical to understanding the mechanism of homologous recombination and the role that certain proteins play in promoting branch migration during recombination. We have recently measured the rate of spontaneous or uncatalysed DNA branch migration. In the presence of physiological concentrations of magnesium, branch migration is quite slow with a step time of about 300 msec. However, in the absence of magnesium, branch migration is significantly faster and, depending on the ionic strength, can proceed 1000 to 10,000 times faster than in magnesium.

We have initiated studies to examine the effect of subtle changes in the conformation of the Holliday junction on the rate of branch migration. Diethylpyrocarbonate and osmium tetroxide have been used to chemically probe the extent of base stacking at the crossover point in synthetic four-way DNA junctions that mimic the Holliday junction. Our results demonstrate that a small drop in the concentration of magnesium ions, from 300 to 100 uM, results in an abrupt loss of base stacking in the four-way junction; this conformational change induced by lowering the magnesium concentration is paralleled by a large increase (about 30-fold) in the rate of spontaneous branch migration. We conclude that some property of the magnesium-induced, stacked Holliday structure presents a kinetic barrier to branch migration; disruption of base stacking at the crossover point circumvents this slow step. Currently, we are employing both enzymatic and chemical modification techniques to assess the conformational changes in the Holliday junction that occur during branch migration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 52016-02 GBB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Thyroid hormone interactions with cells and proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jacob Robbins	Chief, Endocrinology Section	GBB, NIDDK
	Salvatore Benvenga	Courtesy Associate	GBB, NIDDK
	Marcia Phyillaier	Biologist	GBB, NIDDK
	Hans Cahnmann	Scientist Emeritus	GBB, NIDDK

COOPERATING UNITS *(if any)*

D. Rader, Division of Intramural Research, NHLBI; Y. Ito, Division of Intramural Research, NHLBI

LAB/BRANCH

Genetics and Biochemistry

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.7	2.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Drs. Benvenga and Robbins have continued efforts to understand the biochemical basis of thyroid hormone interaction with serum lipoproteins. The T_4 binding domains of the HDL apolipoproteins have common features, including one site per molecule, affinity between serum albumin (HSA) and transthyretin (TTR); relative affinities for T_4 analogs similar to HSA; distinct from the lipid the cell surface receptor binding domains; N-Terminal location. We previously reported sequence homology between the T_4 binding domains of apoA-I, apoE and apoB-100 and have now extended this to other HDL apos, HSA, TTR and TBG using University of Wisconsin Genetics Computer Group and GenePro version 5 programs. Of 10 apo sequences, 5 or more had identical or conserved amino acids in 24/49 positions. The most conserved region was N-terminal in positions 1-26. A conserved motif (Y, L/I/V/M, X, X, V/L/I) occupied positions 22-26. There were 15-16 conserved residues homologous to the apos in HSA, but only 9-11 in TTR and TBG (a 50% difference). A 25 a.a. stretch of HSA, was >40% homologous with the apos. Thus we have shown that there is extensive homology between T_4 binding domains even in distantly related proteins.

Potential physiological importance of the thyroid hormone lipoprotein interactions have been discussed in an invited review in Trends in Endocrinology and Metabolism. Our previous work demonstrated that binding to LDL facilitated the entry of thyroxine into human fibroblasts and was mediated by the apoB/apoE cell surface receptor. Experiments are in progress to extend these observations to human hepatocytes and also to determine whether the lipoproteins are involved in the exit of thyroid hormone from the cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 52017-02 GBB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Thyroid Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jacob Robbins	Chief, Endocrinology Section	GBB, NIDDK
Others:	J. Desiree Pineda	Clinical Associate	GBB, NIDDK
	Marcia Phyillaier	Biologist	GBB, NIDDK

COOPERATING UNITS *(if any)*
J. Reynolds, Nuclear Medicine, CC; M. Merino, Laboratory of Pathology, NCI; C. Meyers, NCI; R. Alexander, Surgery Branch, NCI; D. Fraker, Surgery Branch, NCI; R. Jensen, University of California, San Francisco

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.8	2.5	0.3

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Efforts have continued on methods to improve the management of high risk thyroid cancer. In one, low dose doxorubicin was used as a radiation enhancer and combined with I-131. Eight patients received 24 such treatments and are being evaluated for potential toxicity. No increased toxicity has been detected thus far.

A second method was to give I-131 therapy doses (150 to 300 mCi) to patients with negative diagnostic I-131 scans in whom the presence of cancer was demonstrated by thyroglobulin in blood. In 16 of 17 such patients, post-therapy scans were positive for recurrent or metastatic cancer. The treatment resulted in significant lowering of serum thyroglobulin levels and disappearance of I-131 uptake in lung metastases and other sites.

In collaboration with Dr. Ronald Jensen (Livermore National Laboratory and UCSF), the radiation-induced allelic loss of erythrocyte glycophorin A (MN blood type) was used as a biological dosimeter to quantitate radiation to bone marrow in thyroid cancer patients treated with I-131. The results showed an increase in NO and NN variant erythrocytes in proportion to the amount of bone marrow radiation calculated in the conventional manner. Since the effect is cumulative and persistent, it can be used in the evaluation of patients who have received I-131 therapy.

In collaboration with Bruce Weintraub, the development of methodology to utilize recombinant human TSH to stimulate I-131 uptake and thyroglobulin release in thyroid cancer is continuing. This will replace the current requirement for patients to become hypothyroid in order to test for residual or recurrent cancer. The multi-institutional Phase III data are now undergoing independent review prior to submission to the FDA for approval.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 52019-02 GBB

PERIOD COVERED

October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular biology of thyroid hormone receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Vera Nikodem	Chief, Mech Gene Reg Section	GBB, NIDDK
Others:	Hideaki Tomura	Visiting Fellow	GBB, NIDDK
	Josef Lazar	Visiting Fellow	GBB, NIDDK
	Marcia Phyillaier	Biologist	GBB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

Mechanisms of Gene Regulation Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.7	2	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of the study described in this report was to define the mechanism underlying quantitatively different thyroid hormone dependent transcriptional activation mediated by thyroid hormone receptors β1 and β2. We have shown that thyroid hormone receptor β2, which differs from β1 in the amino acid composition of the amino-terminal region, transactivates malic enzyme thyroid hormone response element (direct repeat + 4) and myelin basic protein thryoid hormone response element (inverted palindrome) poorly, when compared with the transactivation mediated by the β1 or α1 receptor. This is not due to different levels of β2 and β1 expression or the inability of β2 to bind to thyroid hormone response element. However, thyroid hormone receptor β2 cannot interact with TFIIB as efficiently as the thyroid hormone receptor β1. Thus, we concluded that the amino terminal regions of thyroid hormone receptor β2 does not affect the receptor binding to thyroid hormone response element; however, it is required in communicating information via TFIIB to the basal transcription machinery, although interactions with other basal transcriptional factors cannot be excluded.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 52020-02 GBB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of Anteroposterior Patterning in Early Frog Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Sheryl Sato	Senior Staff Fellow	GBB, NIDDK
Others:	Song Huang	Visiting Fellow	GBB, NIDDK
	Samir Witta	Visiting Fellow	GBB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH Genetics and Biochemistry Branch

SECTION Mechanisms of Gene Regulation

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS: 3.1	PROFESSIONAL: 3.0	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

POU transcription factors have been shown to be important in the regulation of cell fate decisions during development. We have focused on understanding the function of one POU gene, XlPOU 2, in early neural development. This gene is first expressed in Spemann's organizer at the beginning of gastrulation, making it one of the earliest neural-specific transcription factors detected. Its temporal and spatial pattern of expression suggests that it may be regulatory in the neural inductive process. To test this possibility, we have misexpressed XlPOU 2 by injecting synthetic XlPOU 2 mRNA into specific blastomeres of the 32-cell stage embryo. As a result of this perturbation we have observed a switch in cell fate of the epidermal lineage towards a neuronal phenotype both in intact embryos, and in vitro, in uncommitted ectoderm. These experiments suggest that XlPOU 2 has a role as a proneural gene in neurogenesis, and is involved in the initial decision ectodermal cells must make in becoming either epidermal or neuronal. We have also identified several novel POU genes in zebrafish, and have begun characterizing a Brain-1 related gene, Brn-1r. This gene is 72% homologous with mouse Brn-1 at the amino acid level. Unlike Brn-1, whose expression is restricted to the developing nervous system, Brn-1r is a maternal transcript. During gastrulation, Br-1r is expressed in the dorsal portion of the embryo, in involuting mesoderm. At later stages, Br-1r expression is prominent in the nervous system. Functional studies are underway to determine whether this gene is involved in early patterning in the embryo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 52021-02 GBB

PERIOD COVERED

October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mapping of triiodothyronine responsive genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Vera Nikodem	Chief, Mech Gene Reg Section	GBB, NIDDK
Others:	Qianxun Xiao	Visiting Fellow	GBB, NIDDK
	Susan Ohlsen	IRTA	GBB, NIDDK
	Roland Lippoldt	Special Volunteer	GBB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

Mechanisms of Gene Regulation Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.2	2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. The steroid-thyroid hormone receptor superfamily comprises a class of ligand activated transcription factors. Ligand activated members of the superfamily include the receptors for steroids, retinoids, thyroid and vitamin D hormones. There is, however, within this superfamily a group of orphan receptors sharing the structural features of nuclear receptors but lacking ligand specificity. We investigated potential functions of two related orphan receptors Nur 77 and Nurr 1 during rat brain development. We cloned two forms of Nurr 1 from a neonatal mouse brain cDNA library; Nurr 1-long identical to that previously reported and a new form termed Nurr 1-short, generated by alternative splicing. Using specific probes from the most dissimilar amino-terminal regions of Nurr 1 and Nur 77 and Northern blot analyses revealed that Nurr 1 mRNA is detectable not only in embryo and during brain development, but also in adult brain, contrary to the previous report. Nur 77 mRNA was already detectable 8 days after birth and gradually increased to day 18 and then stayed unchanged through the adult life. Our preliminary results using a digoxigenin labeled probe indicate that Nur 77 mRNA are expressed in manay regions of adult brain, while Nurr 1 mRNA is more restricted especially to sensory neurons. A long term goal is to target Nurr 1 and Nur 77 genes by homologous recombination technique to determine their physiological roles during rat brain development.

2. We have utilized a differential mRNA display technique to find differences in a subset of mRNA samples prepared from undifferentiated a glial precursor cell line CG4 which differentiate into oligodendrocytes in the absence of mitogens. Several unique messages have been recovered and they are being characterized in a search for genes involved in differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 52022-02 GBB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Protein Entry into the Secretory Pathway

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Harris Bernstein	Senior Staff Fellow	GBB, NIDDK
Others:	Nancy Ulbrandt	IRTA	GBB, NIDDK
	John Newitt	IRTA	GBB, NIDDK
	Niloofar Eskandari	Summer IRTA	GBB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH	Genetics and Biochemistry Branch
SECTION	Molecular Genetics Section
INSTITUTE AND LOCATION	NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS: 2.6	PROFESSIONAL: 2.5	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to understand in detail how newly synthesized proteins which must be secreted from cells or inserted into biological membranes are identified and directed to transport sites in the endoplasmic reticulum (ER) or the cytoplasmic membrane (in prokaryotic cells). The project combines biochemical and genetic approaches to investigate the role of the signal recognition particle ribonucleoprotein (SRP), the signal recognition particle receptor (SRP receptor), and their bacterial homologs in this process. Previous studies have indicated that SRP and the SRP receptor are key components of the cellular machinery responsible for the "initiation" phase of protein export. We expect that in addition to augmenting our understanding of protein sorting, this project will provide insight into the regulation of multi-step pathways, the function of proteins that have broad substrate specificies, and the functional capabilities of RNA.

Previous work indicated that the "signal sequences" which earmark proteins for entry into the ER bind to a site in the COOH-terminal domain of the 54kd subunit of mammalian SRP (SRP54). Recent experiments, however, suggest that sequences in the NH2-terminal domain, which contains a GTPase activity, might also play a role in signal sequence binding. We have introduced point mutations into a conserved motif in the NH2-terminal domain of SRP54 which does not correspond to sequences found in any other GTPase. The mutations do not affect GTP binding or hydrolysis, but rather reduce signal sequence binding activity. We are now examining the mutants in established biochemical assays to determine the mechanism by which sequences in the NH2-terminal domain of the protein participate in signal sequence binding.

Because of methodological limitations to studying the SRP targeting pathway in mammalian cells, we have begun to study homologs of SRP and the SRP receptor in E. coli. The ease of performing genetic experiments in this organism is one reason that it is an attractive model system. We have used both site-directed mutagenesis and phenotypic selection to obtain mutants of homologs of SRP54 ("Ffh"), SRP RNA ("4.5S RNA") and the SRP receptor ("FtsY") which have distinctive phenotypes. We are using these mutants as tools to study the SRP pathway in detail. Genetic analysis of Ffh has supported our previous proposal that conserved methionine residues in its COOH-terminal domain play a role in signal sequence binding and has provided evidence that it functions as part of a complex with 4.5S RNA. Cell fractionation studies have also indicated that a majority of Ffh molecules are associated with membrane bound polysomes, consistent with the notion that like eukaryotic SRP54, Ffh has a targeting function in E. coli.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 52023-02 GBB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genes ancestral to the thyroid/steroid receptor family

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Joseph E. Rall	Senior Scientist	GBB, NIDDK
Others:	Zdenek Kostrouch	Visiting Associate	GBB, NIDDK
	Marta Kostrouchova	Special Volunteer	GBB, NIDDK

COOPERATING UNITS (if any)

University of Acquila, Italy

LAB/BRANCH Genetics and Biochemistry Branch

SECTION Molecular Genetics Section

INSTITUTE AND LOCATION NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF-YEARS: 3.1	PROFESSIONAL: 3	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project is to determine the earliest phylogenetic occurrence of genes of the Thyroid/Steroid hormone receptor family in order better to understand their function. More than thirty members of this gene family are known but there are few data on their occurrence in primitive forms. Many of these gene products, although they possess a ligand binding domain have no known ligands. To obtain clones we have used degenerate primers involving the DNA binding domain which is well preserved in this family, and have devised procedures including PCR to allow efficient cloning of the relevant genes. We have previously isolated a sequence for medusa but have so far been unable to get a full length cDNA. We isolated three clones from ciona intestinalis and Dr Janinni in Italy is currently working on one of them. We have now isolated and completely sequenced three cDNAs from C elegans (a primitive round worm). One of these clones (ce3) shows highest amino acid homology to DHR 3, one of the ecdysone response genes in drosophila. Another, ce8 shows lesser homology to an early human response gene(nak1), and a low but significant homology to NOT1, a yeast general negative regulator of transcription. Ce14 is most homologous to the retinoic acid receptor gene. All the homologies outside the DNA binding domain are so low that the genes we describe appear to be new members of this family. Northern analysis shows that ce3 and ce8 are highly expressed in embryos and in larval stages. All three genes are expressed to about the same extent in adults. In preliminary experiments with in situ hybridization using a digoxigenin labelled riboprobe and an antibody conjugated with alkaline phosphatase, results similar to the Northern experiments were obtained.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DK 53100-06

PERIOD COVERED
October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Identification and characterization of receptors for GI peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.T. Jensen	Branch Chief	DDB, NIDDK
Others:	S.A. Mantey	Chemist	DDB, NIDDK
	R. Benya	Clinical Associate	DDB, NIDDK
	T. Kusui	Visiting Associate	DDB, NIDDK

COOPERATING UNITS *(if any)*
Tulane University, New Orleans, LA (D.H. Coy).

LAB/BRANCH
Digestive Diseases Branch

SECTION
Gastroenterology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.1	2.1	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

I. Identification/characterization of receptors for GI peptides.

I.A. Nature and role of glycosylation of receptors for bombesin-related peptides [mouse gastrin-releasing peptide receptor (mGRP-R) and rat neuromedin B receptor (rNMB-R)]. Using cross-linking, specific glycosidases and functional studies after deglycosylation, the mGRP-R and rNMB-R were shown to be 50 and 35% glycosylated respectively; and to be N-linked complex oligosaccharides. The rNMB-R but not the mGRP-R had terminal sialic acids. All extracellular potential glycosylation sites on both receptors were glycosylated with the mGRP-R having 4 and the rNMB-R having 2. Glycosylation was essential for mGRP-R, but not for rNMB-R G-protein-coupling and high affinity binding. These studies will be coupled with site-directed mutagenesis studies in the future to determine which residues in the GRP-R are important in mediating the high affinity state.

I.B. Characterization of GI peptide receptors by development of specific antagonists.

1. GRP-active configuration. Structure-function studies using conformationally restricted amino acid substitutions in GRP were performed in collaboration with Prof. D.H. Coy (Tulane University) and provided evidence to support a putative folded conformation of the receptor bound Bn and the importance of the His^{12} in receptor activation.

2. Identification of novel VIP receptor antagonists. The physiological role of the ubiquitous neurotransmitter peptide VIP remains unclear because of the lack of specific receptor antagonists. We applied two strategies used successfully with related peptides. In collaboration with Dr. D.H. Coy, Peptide Research Labs, Tulane University, pseudopeptides of the biologically active NH_2 terminus and chimeric peptides were made. Neither approach yield potent antagonists, however VIP (6-28) was found to be 2 times more potent than existing antagonists and should be useful as a starting point to modify to attempt to develop more potent antagonists.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53101-06

PERIOD COVERED

October 1, 1993, to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular basis of action of gastrointestinal peptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.T. Jensen	Branch Chief	DDB, NIDDK
	R.V. Benya	Clinical Associate	DDB, NIDDK
	Y. Kitsukawa	Visiting Fellow	DDB, NIDDK
	J. Mrozinski, Jr.	Chemist	DDB, NIDDK

COOPERATING UNITS (if any)

National Institute of Dental Research, Clinical Investigations Branch, NIH (R.J. Turner); Department of Surgery, University of Cincinnati (R. Bell); Dept. of Pathology, Dartmouth Univ., (Longnecker).

LAB/BRANCH

Digestive Diseases Branch

SECTION

Section on Gastroenterology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.6	2.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

V.1. Gastrin-releasing peptide receptor activation: role of cAMP. GRP-R activation results in both increases in cAMP and phospholipase C (PLC). The mGRP-R stably transfected into BALB-3T3 cells behaved in an identical fashion to the wild type on Swiss 3T3 cells, for binding, G-protein coupling, PLC activation and receptor modulation (internalization, down-regulation, desensitization), however it did not cause increases in cAMP, growth or c-fos mRNA. Detailed studies of both cell types demonstrate increases in cAMP do not mediate GRP-R modulation, but are important in bombesin-induced growth.

V.2. Role of calcium in pepsinogen secretion. To define the roles of $[Ca^{2+}]$ in pepsinogen release, the effects of thapsigargin, CCK and secretin were compared in dispersed chief cells. Our results demonstrate that: 1) increases in $[Ca^{2+}]_i$ due to Ca^{2+} influx caused by TG or CCK-8 can result in pepsinogen secretion. 2) mobilization of Ca^{2+} from intracellular sources is important in the rapid initial phase of pepsinogen secretion caused by CCK-8 and 3) the increase in $[Ca^{2+}]$ per se whether caused by mobilization from $[Ca^{2+}]_i$ stores or Ca^{2+} influx, is responsible for CCK-8 or TG potentiating secretin-stimulated pepsinogen release.

V.3. Action of somatostatin (SS) on chief cells. We demonstrated for the first time that chief cells possess high affinity SS receptors likely of the $SSTR_1$ subtype by binding studies. SS receptor occupation was regulated by agents that activate PKC or adenylate cyclase. SS receptor activation decreased the activity of adenylate cyclase but had no effect on pepsinogen release by various secretagogues.

V.4. Distinguishing multiple CCK receptor subtypes. Using dispersed guinea pig chief cells and COS-7 cells transiently transfected with human $hCCK_A$-R or $hCCK_B$-R, recently described CCK receptor subtype selective agonists and antagonists are demonstrated to be useful in both species for distinguish the role of each CCK receptor subtype in different physiological processes.

V.5. Subtype of CCK receptor mediating CCK-induced pancreatic growth. CCK stimulates carcinogen-induced tumor formation and growth of the pancreas. In collaboration with Drs. R.H. Bell, Univ. of Cincinnati, and D.S. Longnecker, Dartmouth Medical School, the question of which subtype of CCK receptor mediated these effects was addressed. The results demonstrate CCK interaction with CCK_A receptors, not CCK_B receptors, mediate this pancreatic growth effect in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53200-03 DDB

PERIOD COVERED

October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Management of islet cell tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.T. Jensen	Branch Chief	DDB, NIDDK
Others:	D.B. Strader	Clinical Associate	DDB, NIDDK
	M. Orbuch	Clinical Associate	DDB, NIDDK
	C. Termanini	Clinical Associate	DDB, NIDDK

COOPERATING UNITS *(if any)*

National Cancer Institute, Surgery Branch, NIH (D. Fraker); Radiology Department, Clinical Center, NIH (J.L. Doppman); Pathology, NCI (Dr. Stetler-Stevenson); Biostatistics and Data Management, NCI, (D. Venzon).

LAB/BRANCH

Digestive Diseases Branch

SECTION

Clinical Investigation Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.1	3.1	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

III. Management of islet cell tumors.

III.A. Studies related to tumor localization, surgery, antitumor treatment .

III.A.1. Possible benefit of surgical resection in gastrinoma patients. Controversy exists whether patients with ZES should undergo surgical exploration. In collaboration with the Surgical Metabolism Section and Data Management Section, NCI, the longterm courses of patients with ZES who did and did not undergo surgical exploration for possible cure were analyzed. A significantly lower ($p<0.003$) rate of development of hepatic metastases were found for the first time in patients undergoing exploration. These results support the need for surgery in gastrinoma patients and because of their similar growth behaviors, other islet-cell tumors.

III.A.2. Possible lymph node primary gastrinomas. It remains unclear whether gastrinomas can originate in lymph nodes. To address this issue, the longterm follow-up of 21 patients with ZES who had only lymph node gastrinomas was analyzed. Longterm (up to 10 years mean - 5 years), 40% remained cured supporting the proposal this disease may arise ectopically in lymph nodes.

III.A.3. Flow cytometry in ZES. In a collaborative study with the Pathology Department (NCI) flow cytometry results of gastrinomas were shown to correlate independently with the disease extent.

III.A.4. Diagnosis of the MEN-I syndrome in patients with gastrinomas. It is currently widely held that patients with MEN-I and ZES are easy to distinguish clinically from sporadic cases. A cohort analysis showed this is untrue and that up to 30% of patients with ZES and MEN-I present with the ZES and can not be distinguished from sporadic cases. All patients with ZES thus require frequent testing for MEN-I and eventually genetic testing.

III.A.5. Use of interferon in ZES. Interferon was shown to have limited efficacy in the treatment of metastatic gastrinomas.

III.B. Control of gastric acid hypersecretion in patients with Zollinger-Ellison syndrome (ZES).

III.B.1. An analysis of 10-years of continuous treatment with omeprazole in patients with ZES was reported. It was concluded the drug is safe and effective even with prolonged usage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 53201-05

PERIOD COVERED
October 1, 1993, to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Receptors on gastric smooth muscle cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.T. Jensen	Branch Chief	DDB, NIDDK
Others:	T. Pradhan	Chemist	DDB, NIDDK
	Z-F. Gu	Visiting Fellow	DDB, NIDDK

COOPERATING UNITS (if any)
Peptide Research Lab, Tulane University, (Drs. Rossowski and Coy).

LAB/BRANCH
Digestive Diseases Branch

SECTION
Section on Gastroenterology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

IV. Receptors on gastric smooth muscle cells.

IV.1. Somatostatin (SS) receptor subtypes on gastric smooth muscle cells. Gastric smooth muscle cells are shown to possess high affinity SS receptors. Smooth muscle cells rapidly degraded SS-14, however with appropriate protein inhibitors present and the use of a peptidase resistant ligand the pharmacology and stoichiometry of the SS receptors were explored. The receptor exists in 2 affinity states which are regulated by a G-protein. In collaboration with Dr. D.H. Coy (Tulane University) using SS receptor subtype specific analogues, the action of SS on these cells was shown to be mediated by the SS-TR-3 subtype.

IV.2. Gastric smooth muscle cells possess two classes of endothelin (ET) receptors, however, only one alters contraction. Gastric smooth muscle cells were shown to possess both ET_A and ET_B receptors, however in contrast to the pancreatic cells, they were not regulated by agents that activate PLC. Only occupation of the ET_A receptor altered contractile behavior.

IV.3. Gastric smooth muscle cells possess high affinity galanin receptors which mediate relaxation by activating adenylate cyclase. Galanin was shown to have a direct effect on gastric smooth muscle cells causing relaxation. By binding studies, high affinity galanin receptors were characterized, occupation of which by agonists activated adenylate cyclase. Structure-function studies showed the N-terminus of galanin determines receptor affinity and that this affinity is regulated by guanine nucleotide binding proteins.

IV.4. Galanin receptor subtypes mediating relaxation. In collaboration with Dr. D.H. Coy (Tulane University) 13 NH_2, COOH and mid-molecule galanin fragments were investigating for their abilities to function as galanin receptor agonists or antagonists. It is concluded that gastric smooth muscle cells possess a unique galanin receptor subtype, and these results suggest 3 not 2 subtypes of galanin receptors likely exist.

V.5. Mechanism of galanin-induced relaxation. Recently, GI smooth muscle cell relaxants have been shown in some cases to both activate adenylate cyclase and NO synthetase. In isolated guinea pig gastric smooth muscle cells both VIP and galanin caused equipotent relaxation. Using NO synthetase and PKA inhibitors our results demonstrate galanin's relaxant effect is mediated only by cAMP whereas VIP both activates adenylate cyclase and nitric oxide synthetase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 53202-03

PERIOD COVERED
October 1, 1993, to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular characterization of receptors for GI peptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.T. Jensen	Branch Chief	DDB, NIDDK
Others:	S.A. Wank	Senior Investigator	DDB, NIDDK
	J. Pisegna	Clinical Associate	DDB, NIDDK
	R. Benya	Clinical Associate	DDB, NIDDK
	S. Pope	Microbiologist	DDB, NIDDK
	S. Poirot	Visiting Fellow	DDB, NIDDK
	J. Mrozinski	Chemist	DDB, NIDDK

COOPERATING UNITS (if any)
K. Huppi, LG, MGS, NCI; J.F. Battey, LBC, DTP, DCT, NCI, J.D. Gardner, St. Louis University, Department of Medicine.

LAB/BRANCH
Digestive Diseases Branch

SECTION
Cell Biology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.7	5.7	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

II. Molecular characterization of GRP receptors.

II.A. Most G protein-coupled receptors have an ARG in the 2nd intracellular loop and an ALA in the distal 3rd loop that are thought important in G-protein coupling. To investigate the cellular basis of GRP-R internalization, R139G and A263E GRP-R mutants were made. Neither activated phospholipase C (PLC), although both bound ligand with high affinities. Using these GRP-R mutants, GRP-R internalization was shown to be mediated by both PLC-dependent (50%) and independent (50%) processes and both required activation of G protein.

II.B. Molecular characterization of receptors for CCK/gastrin-related peptides.

II.B.1. Chromosomal localization of CCK_A and CCK_B receptors in mouse and human. The gene encoding the CCK_A-R maps to a syntenic region of human chromosome 4 and mouse chromosome 5. The CCK_B-R is on a syntenic region of human chromosome 11 and distal mouse chromosome 7.

II.B.2. Demonstration of 3 states of the CCK. Using the cloned CCK_A-R expressed in COS-7 cells and dispersed pancreatic acini, the CCK_A-R was shown to exist in 3 affinity states rather than 2 states as previously widely-believed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK 53501-18 DDB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies Relating to Pathogenesis of Hepatic Encephalopathy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	A.M. DiBisceglie	Chief	LDS, NIDDK
Others:	J. Vergalla	Chemist	LDS, NIDDK
	M.G. Swain	Guest Researcher	LDS, NIDDK
	C. Yurdaydin	Visiting Associate	LDS, NIDDK
	N.V. Bergasa	Senior Staff Fellow	LDS, NIDDK

COOPERATING UNITS (if any)

Laboratory of Neuroscience, NIDDK (P. Skolnick and A.S. Basile), Mental Health, DIR, NIMH (S.M. Paul), National Institute of Drug Abuse, Baltimore (R.B. Rothman)

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Both clinical and electrophysiologic (VER waveform) ameliorations of hepatic encephalopathy (HE), have been induced in animals with FHF by benzodiazepine (BZ) receptor ligands with antagonist properties. Furthermore spontaneous in vitro activity of Purkinje neurons from rabbits in HE due to FHF exhibited increased sensitivity to depression by agonists of the GABA/BZ receptor complex, including a BZ, and in contrast to control neurons, exhibited excitation when exposed to BZ receptor antagonists. In addition a BZ receptor antagonist reversed the hypersensitivity of HE rabbit neurons to depression by a GABA agonist. The functional status of the chloride ionophore of the GABA/BZ receptor complex has been shown to be normal in a rat model of HE due to FHF. Radioligand binding to BZ receptor determined autoradiographically, was decreased in thin unwashed sections from HE rabbit brains. Purification and characterization of HE ratbrain extracts revealed the presence of reversible, competitive, BZ receptor ligands with agonist properties. Two of the ligands have been chemically characterized as the 1,4-BZs diazepam and N-desmethyldiazepam. The concentrations of the compounds were 2-9 fold greater in HE rat brain than control brain. Overall, these findings suggest that in HE due to FHF: (i) There is increased GABAergic tone; (ii) Blockading of BZ receptors can ameliorate HE; (iii) BZ receptor antagonists may be of value in the management of HE; and (iv) Endogenous BZ receptor ligands in ameliorating HE in animal models does not appear to depend on their affinity for BZ receptor subtypes in addition to the dizawpam sensitive receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53511-13 DDB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Trials of Therapies for Primary Biliary Cirrhosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	J.H. Hoofnagle	Director,	DDDN, NIDDK
Others:	N.V. Bergasa	Senior Staff Fellow	LDS, NIDDK
	A.M. DiBisceglie	Chief	LDS, NIDDK
	M.G. Swain, S.C. Chia	Guest Researcher	LDS, NIDDK
	H. Conjeevaram	Guest Researcher	LDS, NIDDK
	R. Sallie	Visiting Associate	LDS, NIDDK
	C. Yurdaydin	Visiting Associate	LDS, NIDDK

COOPERATING UNITS (if any)

Laboratory of Experimental Immunology, NCI, Dr. David Adams

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
3.3	3.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Primary biliary cirrhosis (PBC) is a progressive liver disease believed to be of autoimmune nature. Its etiology is unknown. It is characterized by progressive intrahepatic cholestasis as a result of ongoing non-suppurative destructive cholangitis that affecting small intrahepatic bile ducts. Several trials have been conducted to study the effect of immunosuppressants in PBC including methotrexate (MTX). Ten patients with symptomatic PBC were treated in an open label pilot study or oral MTX (15 mg/week). Two remain on therapy after 3 and 5 years respectively, while the drug was stopped in the remaining patients after 1 to 2 years because of apparent lack of effect. A trial that studies the effect of 7.5 vs 15 mg per week of oral MTX was started. Patients were randomized to receive one of the two doses and stratified according to the presence or absence of symptoms. 19 symptomatic and 10 asymptomatic patients have been entered into this trial.

Preliminary observations indicate that patients with advanced disease (stages III or IV) do not seem to respond to MTX. Patients with earlier stages (I, II and "early" III) appear to have a better response with a decrease in the activity of serum aminotransferases and alkaline phosphatase and histological improvement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK 53516-03 DDB

PERIOD COVERED

October 1, 1993 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Opiate System in Cholestatic Liver Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	A.M. DiBisceglie	Chief	LDS, NIDDK
Others:	N.V. Bergasa	Senior Staff Fellow	LDS, NIDDK
	J. Vergalla	Chemist	LDS, NIDDK
	M.G. Swain	Guest Researcher	LDS, NIDDK
	C. Yurdaydin	Visiting Associate	LDS, NIDDK
	R. Sallie	Visiting Associate	LDS, NIDDK

COOPERATING UNITS (if any)

NHLBI (Dr. S. Sabol, Dr. W. Scott Young), DIR, NIAID (Dr. David Alling), Biomedical Engineering (Thomas Talbot and Eli Walker)

LAB/BRANCH

Digestive Disease Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The hypothesis that increased opiodergic neurotransmission contributes to the pathophysiology of cholestasis, and in particular to the pruritus associated with this syndrome was tested. In a double-blind placebo controlled trial of 29 patients with pruritus from cholestatic liver diseases, naloxone infusions were associated with decreased scratching activity and decreased perception of pruritus (as assessed by a visual analogue scale). The use of the oral opioid antagonist nalmefene had also been evaluated in 17 patients with this form of pruritus, and was found to be associated with decreased scratching activity and visual analogue score (VAS). Based on these findings, a double-blind placebo controlled trial of nalmefene was conducted in 11 patients with the pruritis of cholestasis. Nalmefene was associated with a 75% reduction of scratching activity and an 86% reduction in the VAS, cholestasis and suggest that nalmefene may be useful on the treatment of this form of pruritus. Adult cholestatic livers from rats with cholestasis secondary to bile duct resection express preproenkephalin (ppENK) mRNA in the proliferating bile ductules and appear to make endogenous opioids de novo. to further study the status of the opioid system in cholestasis the content of proenkephalin-derived endogenous opioids was measured in bileduct resected livers and in controls.

Cholestatic livers had significantly higher concentration of endogenous opioids suggesting that they accumulate in the liver in cholestasis and most likely, synthesize them de novo. ppENK mRNA was sought in the livers of patients with PBC in baseline liver biopsies and during methotrexate treatment by the use of a solution hybridization assay. No ppENK mRNA was found.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 54001-03 DDB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies of the Natural History and Treatment of Chronic Type B Hepatitis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Adrain M. DiBisceglie	Chief	LDS, NIDDK
Others:	J.H. Hoofnagle	Director	DDDN, NIDDK
	M.W. Fried, H. Conjeevaram	Staff Fellow	LDS, NIDDK
	R. Sallie	Visiting Associate	LDS, NIDDK
	M. Battegay, A. Mangia	Guest Researcher	LDS, NIDDK
	M. Shindo, Y-W. Chung	GUest Researcher	LDS, NIDDK
	M. Beames	Lab Technician	LDS, NIDDK

COOPERATING UNITS (if any)
E. Tabor, DCE, NCI, S. Straus, LCI, NIAID. J. Gerin, Georgetown University.
S. M. Feinstone, CBER, FDA.

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.8	5.3	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A cohort of patients with chronic type B hepatitis is being evaluated and folowed prospectively to determine the long-term natural history of this common form of chronic liver disease. Selected patients have been entered into therapeutic trials in which antiviral or immunodulatory agents are being administered. Several agents have been evaluated including alpha interferon, ribavirin, and fluoro-iodo arabinofuranosyl-uracil (FIAU).

Alpha interferon continues to be evaluated in atypical or unusual patients who would ordinarily excluded from controlled trials including those with decompensated cirrhosis, extra-hepatic complications of hepatitis B and atypical serologic markers. A controlled trial of interferon in children with hepatitis B is underway. To date, xx patients have been enrooled. None have completed the study. Two pilot studies of FIAU, a nucleoside analogue, have been conducted. In the first, dose-finding, study 24 patients were treated FIAU at doses of 0.05, 0.1, 0.25 and 0.5 mg/kg/day orally for 4 weeks. FIAU therapy was associated with marked suppression (85% decrease) of serum HBV DNA levels after 4 weeks of therapy. Levels of HBV DNA often remained suppressed for prolonged periods after stoping FIAU. Two patients cleared hepatitis B e antigen from serum; hepatitis subsequently relapsed in one of these patients. In a second study of FIAU, designed for patients to receive therapy for 6 months, 15 patients eneterede the study. Severe side effects of the drug became apparent in 7 patients after taking the drug for 8 to 12 weeks. These patients appeared to develop a Reye's-like syndrome which is currently being investigated further. This study was discontinued because of toxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 54002-03 DDB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Natural History and Treatment of Chromic Type C Hepatitis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Adrain M. DiBisceglie	Chief	LDS, NIDDK
Others:	J.H. Hoofnagle	Director	DDDN, NIDDK
	M.W. Fried, H. Conjeevaram	Staff Fellow	LDS, NIDDK
	R. Sallie, M.G. Swain	Visiting Associate	LDS, NIDDK
	C. Yurdaydin	Visiting Associate	LDS, NIDDK
	M. Shindo, L. Simpson	Guest Researcher	LDS, NIDDK
	K. Mahaney, M. Battegay	Guest Researcher	LDS, NIDDK
	M. Beames	Lab Technician	LDS, NIDDK

COOPERATING UNITS (if any)

H.J. Alter, Dept. Transfusion Medicine, CC, NIH. S.M. Feinstone, CBER, FDA.
K. Krawczynski, Hepatitis Branch, CDC

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.6	4.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patients with well-documented chronic hepatitis C are being evaluated to determine the long-term natural history of this common form of chronic liver disease. A cohort of such patients are available to evaluate experimental therapies. Previous studies have shown that alpha interferon therapy has a short-term beneficial effect in approximately 50% of sustained in approximately 20% after stopping interferon. A randomized, placebo-controlled trial of ribavirin is currently underway. So far, 32 of 58 patients have completed first year of the study. Prolonged ribavirin administration was associated with significant improvement in serum aminotransferase activities and in hepatic lobular necrosis despite unchanged serum levels of HCV RNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 DK 55000-22 MCEB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biosynthesis, Glycosylation, and Action of Thyrotropin: Clinical Trials of Recombinant TSH

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. D. Weintraub	Chief	MCEB, NIDDK
Others:	N. R. Thotakura	Visiting Scientist	MCEB, NIDDK
	M. Szkudlinski	Visiting Fellow	MCEB, NIDDK
	J. E. Tropea	Postdoctoral IRTA Fellow	MCEB, NIDDK
	J. East-Palmer	Biologist	MCEB, NIDDK
	M. Grossmann	Special Volunteer	MCEB, NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH Molecular and Cellular Endocrinology Branch

SECTION Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 3.5 | PROFESSIONAL: 3.5 | OTHER: 0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recombinant thyrotropin which is used to stimulate radioiodine uptake in patients with thyroid cancer has been produced by novel methods of transfection of alpha and hTSH beta minigenes into Chinese hamster ovary cells. Utilizing products produced in a large scale bioreactor as well as a hollow fiber bioreactor, we have been able to purify several hundred milligrams of this product. Terminal sialylation of recombinant TSH determines in vitro and in vivo bioactivity as well as clearance rate. The carbohydrate structure of recombinant TSH has been characterized in detail and differs from pituitary TSH. The role of sugar structures in bioactivity has also been clarified by enzymatic deglycosylation.

Site-directed mutagenesis as well as production of novel recombinant hybrids has been used to develop new TSH analogs with prolonged half life. We have also developed competitive antagonists of TSH which will be useful in treatment of patients with hyperthyroidism and thyroid cancer.

U.S. GOVERNMENT PRINTING OFFICE. 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 55002-14 MCEB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology of Pituitary Glycoprotein Hormones and Hypothalamic Releasing Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. D. Weintraub	Chief	MCEB, NIDDK
Others:	M. K. Kim	Senior Staff Fellow	MCEB, NIDDK
	L. A. Lesoon-Wood	IRTA Postdoctoral Fellow	MCEB, NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH Molecular and Cellular Endocrinology Branch

SECTION Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 2.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The human TSH beta gene is expressed only in the thyrotrophs of the anterior pituitary where it is regulated by hypothalamic hormones, cyclic AMP as well as thyroid hormone. Recent studies have indicated that TRH and cyclic AMP mediate their transcriptional action in large part through changes in phosphorylation of the specific pituitary factor pit-1 which binds to a region upstream of the start of transcription. In contrast, thyroid hormone mediates its inhibition through binding to a regulatory element downstream of the start of transcription in the first untranslated exon.

We have also characterized in detail a TGGGTCA motif at -1/+6 of the hTSH-beta gene that mediates, in conjunction with the upstream pit-1 site, protein kinase C and A as well as TRH stimulation of hTSH-beta gene expression. We have also shown that c-fos and c-jun which interact with this downstream site modulate T3 inhibition of hTSH-beta gene expression.

We have recently recognized other important regulatory sequences for hTSH-beta gene expression. One such area is a pituitary-specific TATAAA sequence at -25 which is vital for both basal and regulated expression. Other regions include unique silencer regions at two locations upstream in the promoter. These regions include Oct-1 binding sites and appear to act as nuclear matrix attachment regions.

US GOVERNMENT PRINTING OFFICE: 1991 0-364-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01DK55006-21MCEB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Insulin-like Growth Factor Binding Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. M. Rechler	Chief, Growth & Development Section	MCEB, NIDDK
	L.A. Bach	Visiting Associate	MCEB, NIDDK
Others:	Y. Boisclair	Visiting Associate	MCEB, NIDDK
	A.L. Brown	Expert	MCEB, NIDDK
	G.T. Ooi	Visiting Associate	MCEB, NIDDK
	D. Suh	Visiting Fellow	MCEB, NIDDK
	L. Tseng	Chemist	MCEB, NIDDK
	Y. Yang	Sr. Staff Fellow	MCEB, NIDDK
	C-Y. Lee	Visiting Fellow	MCEB, NIDDK
	F. Cohen	Med. Staff Fellow	MCEB, NIDDK

COOPERATING UNITS *(if any)*
Holland Laboratory, American Red Cross, Rockville, MD (J.F. Perdue); Molecular Biology Research Laboratory, Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan (K. Sakano); Centro de Investigaciones Biologicas, CSIC, Madrid, Spain (F. de Pablo)

LAB/BRANCH Molecular & Cellular Endocrinology Branch

SECTION Growth & Development Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892-1758

TOTAL MAN-YEARS: 8.00 | PROFESSIONAL: 7.00 | OTHER: 1.00

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued to investigate how insulin-like growth factor binding proteins (IGFBPs) modulate the biological actions of IGF-I and IGF-II. The IGFs occur in plasma, other extracellular fluids, and tissues complexed to one or more members of a family of six IGFBPs. The IGFBPs determine the bioavailability of the IGFs and may inhibit or potentiate IGF action. As they have distinct properties and are differently regulated, the six IGFBPs provide a flexible and versatile regulatory system. The two thrusts of our program in the past year have been: (i) the transcriptional regulation of the rat IGFBP-1 and IGFBP-2 genes, because the synthesis and abundance of these proteins is largely regulated at the transcriptional level; and (ii) the biological actions of IGFBPs on target cells, using recombinant IGFBP-6. Noteworthy results include: (i) IGFBP-1 promoter. A region has been identified in the proximal promoter that determines the stimulation of promoter activity by cyclic AMP and phorbol esters. In addition, a glucocorticoid response element and an insulin response element have been identified and shown to be essential for the stimulation of promoter activity by dexamethasone, and the rapid inhibition of promoter activity by insulin, respectively. Present efforts are directed toward identifying the transcription factor that binds to the insulin response element, and the signal transduction pathway by which insulin regulates IGFBP-1 transcription. (ii) IGFBP-2 promoter. The IGFBP-2 promoter is GC-rich, but lacks TATA and initiator sequences. Three clustered Sp1 sites in the proximal promoter are critical for basal promoter activity in BRL-3A rat liver cells. We are presently trying to determine the relative contributions of the Sp1 sites and of secondary structure in the proximal promoter region to the choice of a precise transcription initiation site. (iii) Biological actions of IGFBPs on target tissues. We have expressed recombinant human IGFBP-6. Like native IGFBP-6, recombinant human IGFBP-6 is O-glycosylated and has 50-fold greater affinity for IGF-II than IGF-I. Incubation of L6A1 rat myoblasts with recombinant IGFBP-6 inhibited IGF-II-mediated myoblast differentiation. The added IGFBP-6 remains in the culture medium, and does not associate with the cell surface or extracellular matrix. Our results are consistent with the hypothesis that pericellular localization is required for IGF-II : IGFBP-6 complexes to be biologically active. Mutants will be introduced into IGFBP-6 to alter its localization to determine if potentiation of biological activity will result.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 DK55015-06 MCEB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mutations of the Thyroid Hormone Receptor Gene in Patients with Thyroid Hormone Resistance

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B.D. Weintraub, M.D.	Chief	MCEB, NIDDK
Others:	R. Wong	Visiting Associate	MCEB, NIDDK
	M. B. Grace	Predoctoral IRTA Fellow	MCEB, NIDDK
	D. Seto	Senior Staff Fellow	MCEB, NIDDK
	F. Davis	Visiting Associate	MCEB, NIDDK
	D.-W. Gong	Visiting Fellow	MCEB, NIDDK

COOPERATING UNITS (if any)
Dr. S.-y. Cheng, Senior Investigator, Laboratory of Molecular Biology, National Cancer Institute

LAB/BRANCH Molecular and Cellular Endocrinology Branch

SECTION Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 3.0 PROFESSIONAL: 3.0 OTHER: 0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Resistance to thyroid hormone (RTH) is an inherited disease linked to mutations in the beta T3 receptor gene and characterized clinically by the resistance of peripheral and pituitary tissues to the action of thyroid hormone. Of the over 50 different mutations identified in the ligand binding domain of the beta receptor, several were shown to inhibit normal receptor function by a dominant negative mechanism which is likely to mediate the phenotype of this disease. Recent studies have indicated that this dominant negative effect is most likely mediated by competiton of mutant and normal receptor for binding to T3 response elements within various thyroid hormone responsive genes.

We have recently shown that natural thyroid hormone response elements (TRE's) show variation in the dominant negative effect compared to previously employed artificial TRE's. These differences appear to explain the diverse clinical features of RTH. We have recently developed a transgenic model of RTH which should be useful in understanding the function of mutant receptors in vivo and in developing new therapies for the disease. Finally, we have developed novel methods to screen for mutations in the receptor to identify mutations rapidly using automated DNA sequencing. All of these advances should be applicable to other dominant genetic diseases.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 57501-18

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Function of DNA Virus Genomes in Animal Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Roland A. Owens	Senior Staff Fellow	LMCB:NIDDK
Other:	Sirkka R.M. Kyöstiö	IRTA Fellow	LMCB:NIDDK
	Ramani S. Wonderling	IRTA Fellow	LMCB:NIDDK
	Jason D. Vargas	MARC Student	LMCB:NIDDK

COOPERATING UNITS *(if any)*

R. Kotin (MH/NHBLI/NIH, Genetic Therapy Inc.); J. Chiorini, B. Safer, E. Urcelay (MH/NHLBI/NIH); B. Antoni, A. Rabson (Rutgers) N. Chejanovsky (Bet-Dagan, Israel); B. Carter (Targeted Genetics Inc.), M. Weitzman (U. Penn)

LAB/BRANCH

Laboratory of Molecular and Cellular Biology

SECTION

Molecular Biology

INSTITUTE AND LOCATION

NIDDK:NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.7	2.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Adeno-associated virus type 2 (AAV) is a non-pathogenic human parvovirus which is being developed as a gene therapy vector. The AAV *rep* gene open reading frame encodes four overlapping Rep proteins. Rep68 and Rep78 bind to the AAV genome's inverted terminal repeats, have enzymatic activities which are necessary for AAV DNA replication, regulate AAV gene expression, inhibit HIV-1 replication, are required for preferential integration of the AAV genome into a region of human chromosome 19, and inhibit cell division. We have expressed wild-type and mutant Rep proteins from the HIV-1 long terminal repeat promoter in human 293 cell transient transfections or as maltose binding protein fusions in *Escherichia coli*. We have shown that Rep78 and Rep68 can mediate the formation of a complex between the ends of the AAV genome and the chromosome 19 integration site. This work may lead to the development of AAV gene therapy vectors with site-specific integration. We have also demonstrated that the repression of AAV RNA levels by Rep proteins is mediated, at least in part, by a Rep binding site within the p5 promoter region of AAV. Our analysis of mutant Rep proteins has shown that part of the down-regulation by wild-type Rep78 and Rep68 is independent of this binding site. Two mutant Rep proteins were found to be dominant-negative for the DNA helicase activity, which had previously been demonstrated for wild-type Rep78 and Rep68. This observation is consistent with the model that the Rep proteins function as multimers. Our mutant Rep proteins that lack DNA helicase activity cannot efficiently down-regulate a p5 promoter with a mutated Rep binding site or the AAV p19 promoter. Studies with a maltose binding protein-Rep68 fusion have demonstrated a DNA-stimulated ATPase activity and the ability to unwind RNA/DNA hybrids. This apparently non-specific RNA/DNA helicase activity may be involved in the aspect of regulation of the p5 promoter which does not depend on binding to the Rep binding site within the promoter, as well as p19 regulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 57502-21

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hormonal Regulation of Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Takami Oka	Senior Investigator	LMCB:NIDDK
Other:	Robert Moore	IRTA Fellow	LMCB:NIDDK
	Tetsuo Murakami	Special Volunteer	LMCB:NIDDK
	Norio Nonomura	Visiting Fellow	LMCB:NIDDK
	John W. Perry	Biologist (Technician)	LMCB:NIDDK
	Hiroshi Saito	Visiting Fellow	LMCB:NIDDK
	Li-Xin Zhou	Visiting Fellow	LMCB:NIDDK

COOPERATING UNITS (if any)

Dr. Koichi Enomoto, Shimane Medical University, Japan, Dr. Sergio Lavandero, Department of Molecular Biology, University of Chile, Chile, Dr. Osamu Tsutsumi, Department of Obstetrics and Gynecology, Tokyo University, Japan

LAB/BRANCH

Laboratory of Molecular & Cellular Biology

SECTION

Cell Growth and Differentiation Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of our current research is to elucidate the molecular mechanism(s) of tissue- and stage-specific, multi-hormone regulated gene expression using the β-casein gene as a tool. β-Casein is the major milk protein synthesized by the mammary gland during lactation. The expression of the mouse β-casein gene is regulated both positively and negatively by the interaction of various peptide and steroid hormones during mammary gland development. Insulin (I), glucocorticoid (H) and prolactin (PRL) are essential for the induction of casein gene transcription and progesterone inhibits the induction process. To study the molecular mechanism of casein gene expression, we have established a transient expression system. Primary mouse mammary epithelial cells were transfected with a β-casein promoter-CAT (chloramphenicol acetyltransferase) reporter plasmid and its expression was examined in the presence of appropriate hormones. Using this system, we found that approximately 500 nucleotides upstream from the transcription initiation site are sufficient to confer hormone responsiveness to the CAT reporter gene. CAT activity was induced by I, H and PRL and was inhibited by progesterone. Additional studies with a series of 5′- and internal deletions of the promoter indicated that at least three regions, located within bp positions -258/+7, contain DNA elements required for hormonal induction. In order to define the transcription factors mediating the hormonal regulation of β-casein gene transcription, we have analysed the binding of mammary gland nuclear proteins to the casein gene promoter using gel mobility shift assays, DNase I footprinting and methylation interference experiments. We identified at least six DNA elements (I - VI) that bind nuclear proteins in the promoter region required for hormonal induction. The binding of nuclear proteins to the six elements increased in the mammary gland during transition from the virgin to lactating periods. Elements III and V are probably recognized by a mammary gland-specific factor whose appearance is induced by PRL. Element IV corresponds to the consensus sequence for milk protein genes. Transfection analysis showed that this element is important for enhancement of β-casein gene transcription. The binding protein to element IV existed in two forms, one dominant in virgin gland and the other in lactating gland. The appearance of the latter form is induced by I. Our results suggest that the multi-hormonal regulation of β-casein gene transcription involves the interplay of multiple transcription factors whose appearance is controlled by various hormones.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 57503-21

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Lysosomal Transport and Storage Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Frank Tietze

COOPERATING UNITS (if any)

William A. Gahl, Section on Human Biochemical Genetics, NICHD

LAB/BRANCH

Laboratory of Molecular and Cellular Biology

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	-

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Copper is an essential element in biological systems by virtue of its coenzyme function in a number of enzyme systems. Two distinct inherited disorders of copper metabolism in humans have thus far been recognized: Menkes disease and Wilson's disease. Recent successful cloning of the genes for these two diseases suggests that in each case the abnormality is probably associated with defective intracellular trans-membrane copper transport. In particular case of Menkes disease, which is characterized by deficient enzyme copper content, it has been suggested that the specific transport defect may be associated with the microsomal membrane. For this reason, and in view of the lack of any information concerning intracellular copper transport, we have studied the uptake of cuprous copper in a model system composed of purified rat liver microsomal membranes. The use of monovalent copper in this instance is dictated by the probable state of this metal in the cytosol of the intact cell. Although time- and temperature-dependent copper uptake into microsomal vesicles has been observed, interpretation of the results has been hampered by a high background of non-specific, irreversible copper binding. It is conceivable that the use of stripped microsomes, i.e., microsomes free of attached ribosomal RNA, may serve to reduce this unwanted background.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 57504-07

PERIOD COVERED
October 1, 1994 to September 30, 1994

TITLE OF PROJECT [illegible]
Regulation of HIV by AAV

PRINCIPAL INVESTIGATOR [illegible]

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular and Cellular Biology

SECTION
Molecular Biology

INSTITUTE AND LOCATION
NIDDK: NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK [illegible]

This project is combined with Z01 DK 57501.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 57800-03

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Initial Intracellular Events of Steroid Hormone Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	S.S. Simons, Jr.	Chief, Steroid Hormones Section	LMCB:NIDDK
Other:	Suzanne F. Bayly	Visiting Fellow	LMCB:NIDDK
	Clayton D. Collier	PRAT Fellow	LMCB:NIDDK
	David A. Jackson	IRTA Fellow	LMCB:NIDDK
	Kevin J. Modarress	IRTA Fellow	LMCB:NIDDK
	Hisaji Oshima	Visiting Associate	LMCB:NIDDK
	Marc E. Poirot	Special Volunteer	LMCB:NIDDK
	Daniele Szapary	Visiting Associate	LMCB:NIDDK
	Min Xu	Visiting Fellow	LMCB:NIDDK

COOPERATING UNITS *(if any)*
D.P. Edwards (Univ. of Colorado, Denver), W.B. Pratt (Univ. of Michigan Medical School, Ann Arbor), K.R. Yamamato (UCSF, San Francisco), G. Schutz (German Cancer Research Center, Heidelberg, Germany)

LAB/BRANCH
Laboratory of Molecular and Cellular Biology

SECTION
Steroid Hormones Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.8	7.8	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to define the initial, intracellular events of glucocorticoid hormone action and steroid hormone action in general. Such studies are of particular current relevance since steroid hormone receptors are arguably the best understood regulators of eukaryotic gene transcription. The first step of steroid binding to the intracellular receptor is followed by activation of the receptor-steroid complex to DNA/nuclear-binding species that then associates with those nuclear acceptor sites involved in the regulation of transcription of selected genes in specific cells. The thiol groups of glucocorticoid receptors have been proposed to be important determinants of receptor activity and to form complexes with molybdate, which stabilize steroid binding activity and block activation of the receptor-steroid complexes. Nevertheless, we found that thiol groups are not required for the actions of molybdate or of the related metal oxyanions tungstate and vanadate. Likewise, although exogenous thiol-specific agents do alter receptor properties, the structural and functional changes of native glucocorticoid receptors that are associated with steroid binding, activation, and dissociation of heat shock protein 90, were found not to require the thiols of receptor protein. Furthermore, the tertiary structure of the receptor appears to be constituted in a manner that prevents the usually facile rearrangements of any disulfide bond-containing structures that might form. One component that did have a major effect on glucocorticoid receptors was a low molecular weight factor(s) that was previously determined to be required for the DNA binding of a sub-population of activated complexes. We have now found that this factor(s) equally affects the glucocorticoid receptors bound by agonists and antagonists and both progesterone and estrogen receptor complexes. Thus, factor-assisted DNA binding may be a general mechanism for all of the classical steroid receptors, which would offer an intriguing level of control for steroid regulated gene expression. Collectively, our current findings contribute to our long term goal of defining the mechanisms of steroid hormone action at a molecular level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 57801-2

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enzymatic Mechanisms of DNA Replication: The Bacteriophage T4 System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Nancy G. Nossal	Chief, LMCB	LMCB:NIDDK
Other:	Todd Capson	Senior Staff Fellow	LMCB:NIDDK
	Lisa Hobbs	IRTA Fellow	LMCB:NIDDK
	David Wheeler	IRTA Fellow	LMCB:NIDDK

COOPERATING UNITS *(if any)*

Drs. Craig Hyde and Tim Meuser, LSBR/NIAMS, Dr. Stephen Benkovic, Department of Chemistry, Pennsylvania State University, University Park, Pennsylvania

LAB/BRANCH

Laboratory of Molecular and Cellular Biology

SECTION

Section on Nucleic Acid Biochemistry

INSTITUTE AND LOCATION

NIDDK:NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	3.1	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are continuing our study of the *E. Coli* bacteriophage T4 model system for duplex DNA replication in which efficient DNA replication *in vitro* is achieved with purified proteins encoded by T4 phage: T4 DNA polymerase (gene 43), gene 32 DNA helix-destabilizing protein, the gene 44/62 and gene 45 polymerase accessory proteins, the genes 41, 61, and 59 primase-helicase, RNase H, and DNA ligase. *Structure of the T4 DNA replication proteins.* We are collaborating with Tim Meuser and Craig Hyde, NIAMS, to determine the structure of each of the T4 DNA replication proteins using single crystal X-ray diffraction. We have obtained high resolution data (2.5Å) on large single crystals of RNaseH and the gene 59 helicase assembly protein, and are screening heavy atom derivatives for phasing potential. *Assembly of replication proteins on a forked DNA template.* We have constructed substrates with a photoactivatable residue in the fork ahead of a primer to learn how and where the polymerase and polymerase accessory proteins and the gene 61, 41, and 59 protein primase-helicase components assemble at the fork. ATP hydrolysis by the 44/62 complex is required to assemble the 45 protein on a primer in a form in which it can move forward on the single-stranded DNA, as shown by its cross-linking to the fork ahead. Polymerase adds to this complex, occupying a position ahead of the 45 protein, and is cross-linked only when there is no gap between the primer and the fork. *Function and structure of the T4 primase-helicase complex.* We are studying the mechanism by which the gene 59 proteins stimulates DNA unwinding by the 41 helicase, and primer synthesis dependent on both the 41 and 61 proteins. We are probing the physical interactions between these proteins by affinity chromatography, centrifugation, and chemical cross-linking. *T4 RNaseH.* We are using purified T4 RNaseH to determine how primer removal by RNaseH and gap filling by polymerase are coordinated on the lagging strand. During 5′ to 3′ hydrolysis T4 RNaseH removes oligonucleotides of 1 to 5b. The rate of digestion and length of the products are increased by 32 protein. T4 DNA polymerase synthesis from an upstream fragment stimulates RNaseH hydrolysis on the adjacent fragment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 57802-07

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bacteriophage T4 Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Deborah M. Hinton	Research Chemist	LMCB:NIDDK
Other:	Mridula Sharma	Visiting Associate	LMCB:NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular and Cellular Biology

SECTION

Section on Nucleic Acid Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	1	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The expression of different classes of bacteriophage T4 genes (early, middle, and late) is transcriptionally regulated. T4 middle promoters, which have a unique consensus sequence ('MotA box') centered at -30, are transcribed by phage-modified host RNA polymerase in the presence of an activator, the T4 MotA protein. The middle promoter PuvsX has the MotA box at -30 as well as other matches at -35, -51, -70, and -87. DNase I footprints indicate that MotA protects PuvsX from positions -25 to -59 on the template strand and from -19 to -58 on the non-template strand, regions that include the MotA boxes at -30, -35, and -51. However, only the MotA box at -30 is required for activation of transcription. MotA also protects from +40 to +57, a region downstream of the transcription start which does not have a recognizable MotA box. This result suggests that MotA may bind to another motif besides the MotA box. MotA binding to PuvsX is specific, but it is also weak. We assayed the stability of MotA, phage-modified polymerase, or both on PuvsX by observing the resistance of the protein-DNA complex to HindIII, which cleaves within the MotA protected region and near sites where unmodified RNA polymerase contacts *E. coli* promoters. We find that the complex formed in the presence of MotA and polymerase is more stable (half-life of 2.5 min) than that made with polymerase alone (half-life of 1.4 min). Under these conditions, no protection by MotA alone is detected. We speculate that MotA makes unstable contacts at the MotA box at -30 which are then stabilized by its interaction with phage-modified polymerase. Although not encoded by an intron, the T4 SegA protein shares amino acid motifs with a family of fungal and phage proteins found within mobile group I introns. We have purified the SegA protein to homogeneity and find that it has a DNA endonuclease activity that cleaves DNA with a hierarchy of site specificity. PCR analyses indicate that several T-even related phages lack the *segA* gene, and a *segA* amber mutant of bacteriophage T4 that we constructed had no detectable phenotype. Taken together, our results support the hypothesis that the *segA* gene of T4 originated from the insertion of a mobile, endonuclease-encoding DNA into an intergenic region of the T4 genome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK58000-49

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analytical Service and Methodology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Edwin D. Becker	Acting Chief, LAC	LAC/NIDDK
Others:	Herman J. C. Yeh	Research Chemist	LAC/NIDDK
	Lewis K. Pannell	Visiting Scientist	LAC/NIDDK
	Noel Whittaker	Chemist	LAC/NIDDK
	Wesley White	Biologist	LAC/NIDDK

COOPERATING UNITS (if any)

Laboratory of Bioorganic Chemistry, NIDDK; Laboratory of Medicinal Chemistry, NIDDK; Laboratory of Biophysical Chemistry, NHLBI; Laboratory of Experimental Pathology, NCI

LAB/BRANCH

Laboratory of Analytical Chemistry

SECTION

Instrumentation Section

INSTITUTE AND LOCATION

NIH/NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.3	2.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Basic research and service functions are performed by members of the Section. A major mission of the organization involves the instrumental and chemical analyses provided to NIDDK scientists, other NIH laboratories and, to a limited extent, personnel of other government agencies. Instrumental analyses include nuclear magnetic resonance of liquids and solutions; infrared spectroscopy of solids, liquids, and samples introduced by gas chromatograghy; mass spectrometry (with samples introduced by solids probe, gas and liquid chromatography and capillary zone electrophoresis and ionization by electron impact, chemical ionization, electrospray, thermospray, thermabeam, and fast atom bombardment)

Requests for analyses are obtained from many laboratories and Institutes at NIH, as well as some outside organizations. The laboratory in this way acts as an NIH resource and it is supported, in part, by its collaborators in terms of staffing and equipment where appropriate. Some major instruments are jointly funded and shared with other Institutes to avoid costly duplication of facilities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK58012-02

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Application of NMR Spectroscopy in Chemical and Biochemical Analysis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Herman J. C. Yeh Research Chemist LAC/NIDDK

Other: Xiaohong Liu Visiting Fellow LAC/NIDDK

COOPERATING UNITS (if any)

Laboratory of Bioorganic Chemistry, NIDDK; Laboratory of Chemical Physics, NIDDK; Laboratory of Molecular Pharmacology, NCI.

LAB/BRANCH

Laboratory of Analytical Chemistry

SECTION

Instrumentation Section

INSTITUTE AND LOCATION

NIH/NIDDK, Bethesda, MD 20892-0805

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Various NMR techniques have been used to solve the solution structures of a number of molecules. These include polysacharides, metabolite of antiangiogenic drug O-chloroacetylcarbamoyl,fumagillol, acid catalyzed rearrangement products of deoxyoartemisinins, and covalent nucleoside adducts from diol epoxides of polycyclic aromatic hydrocarbons.

We have analyzed 500 MHz NMR NOE spectra of a DNA duplex modified at the adenosine residue with a highly carcinogenic diol epoxide metabolite of benzo[a]pyrene. These NOE data in conjunction with computer spectral simulation and distance restained molecular dynamics calculation indicated that the modified dA5 adopts an unusual syn glycosidic torion angle and that the pyrenyl moiety intercalates between bases of dG13 and dG14 of the complementary strand.

In an attempt to gain insight into the mechanism of the formation of the less receptive isomeric nucleosides by purine nucleoside phosphorylase, a number of N-7 isomers of ribo- and deoxyribo-nucleosides have been synthesized and their reaction products in the presence of the enzyme have been monitored by NMR. This work is in preparation for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK58013-02 LAC

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mass Spectrometry of Drugs, Natural Products, Proteins and Oligonucleotides.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

L.K. Pannell	Visiting Scientist	LAC, NIDDK
P. Lecchi	Visiting Fellow	LAC, NIDDK
Q-l. Pu	Special Volunteer	LAC, NIDDK
Han Le	Stay-in-school	LAC, NIDDK

COOPERATING UNITS (If any)
Daly and Jerina, LBC, NIDDK; Fales, LBC, NHLBI; Basile, LNS, NIDDK; Gustafson, Beutler and Cardellina, NCI, FCRC; Kashman, Tel Aviv Univ., Israel; Munro and Blunt, U. Canterbury, NZ; Peterkofsky, IR, NHLBI; Stein, NIST.

LAB/BRANCH
Laboratory of Analytical Chemistry

SECTION
Structural Mass Spectrometry Unit, Instrumentation Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Specialized mass spectrometry analyses are provided to the laboratory and to other collaborating units. The emphasis of this work is split between identification of trace organic compounds isolated from biological sources and the mass spectrometry of biopolymers (e.g. peptides, proteins and oligonuceotides). A very close working relationship and collaboration is maintained with the Laboratory of Biophysical Chemistry, NHLBI, especially with the research into, and use of techniques for, mass spectrometry of macromolecules. The camera added to the electrospray source on the JEOL SX102 instrument allows for easy optimization of the spray during analyses. A request has been placed to upgrade the electrospray source to enhance stability and sensitivity, and to facilitate interfacing to liquid chromatographic methods. This will be used as part of the Unit's effort into post-translational modifications of proteins. The capillary zone electrophoresis instrument continues to receive heavy usage from this laboratory and others in the Institute. Its separation efficiency far surpasses that of traditional HPLC methods. As part of the macromolecule collaboration with LBC, NHLBI (Dr. Fales), routine use is made of a mass spectrometer especially set up for sequencing of protein digests. In addtion, use is made of their laser desorption mass spectrometer for the analysis of proteins and protein digests as part of our work in biopolymer mass spectrometry. In the small molecule area, collaborative interest has continued in the identification of biologically active natural products, especially those of interest in AIDS research and treatment. Samples analyzed derive from many facilities and researchers and approximately 2800 samples were analyzed on the JEOL alone during the year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK 58501-08 LN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT [illegible]

Receptors for Neurotransmitter & Drugs in Brain & Peripheral Tissues

PRINCIPAL INVESTIGATOR [illegible]

P.I.: P. Skolnick, Ph.D. Chief, LN; Pharmacologist: A.S. Basile, Ph.D.; Staff Fellows: L. Fossom, Ph.D.; Visiting Associates: Y. Li M.D.; Y. Sei M.D., Ph.D.; IRTA Fellows: B.D. Harris, M.D.; C. Kaufman, Ph.D.; R.T. Layer Ph.D.; G. Wong, Ph.D D. Gunnerson Ph.D.; Visiting Fellows: P. Popik, M.D., Ph.D.; L-M Zhou Ph.D.;Predoctoral IRTAs: J. Curtis M.S.; T. Lyon B.S.; Special Volunteers: E. Kustova Ph.D.; K. Bambha M.S.; J. Hee-Ha M.D., Ph.D.; Guest Workers: M. Benveniste Ph.D.; E. Moody M.D.

COOPERATING UNITS (if any)

K.C. Rice, LMC, NIDDK; K. Jacobsen, LBC, NIDDK; D. von Lubitz, LBC, NIDDK; J. Hartley, H. Morse, LI, NIAID; cooperating units continued on page 2.

LAB/BRANCH

Laboratory of Neuroscience

SECTION

Section of Neurobiology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
13.0	13.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK [illegible]

High affinity, stereospecific recognition sites (receptors) for neurotransmitter, neuromodulators, and many clinically useful drugs have been identified in both peripheral tissues and the central nervous system. The interaction of a neurotransmitter, neuromodulator or a drug with a specific recognition site initiates a series of events (for example, the opening of an ion channel or activation of an enzyme) resulting in either a physiological response (in the case of a neurotransmitter or neuromodulator) or a pharmacological effect (in the case of a drug). Furthermore, the presence of recognition sites for synthetic substances indicates that endogenous substances may also be present which can mimic (or antagonize) the effects of exogenously applied (synthetic) compounds. Studies are in progress to characterize "recognition-effector" systems with special emphasis on ligand (transmitter)-gated ion effector systems, to isolate and identify novel endogenous ligands of physiological and pathophysiological importance, to develop appropriate model systems to examine these phenomena, and to relate recognition-effector systems to both physiological and pathophysiological processes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 59,501-08 LMC

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Design and Synthesis of Drugs Acting on Central and Peripheral Tissues

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. C. Rice	Chief, Drug Design & Synth. Section	LMC-NIDDK
Co PI:	A. E. Jacobson	Deputy Chief, LMC	LMC-NIDDK

Others: P. Fleming, J. Pinto, D. Lewis, Y. Zhang, IRTA; S. Calderon, D. Matecka, Visiting Fellows; H. Kayakiri, E. Ohshima, SV. All LMC, NIDDK.

COOPERATING UNITS (if any)

LN-NIDDK (P. Skolnick); U Arizona (F. Porreca); U of Michigan (J. Woods, C.P. France); CC-NM (D. Kiesewetter, M. Channing); U Alabama (E. Blalock); BPB-NIMH (A. Pert); Research Triangle Institute (F.I. Carroll); ARC, NIDA (J.L. Katz, R.B. Rothman, H.C. Akunne); NCI (T.R. Burke Jr.).

LAB/BRANCH

Laboratory of Medicinal Chemistry

SECTION

Drug Design and Synthesis Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 9.0	PROFESSIONAL: 9.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Synthetic programs are continuing to develop new ligands for imaging brain drug receptors by positron emission tomography (PET) and single photon emission computed tomography (SPECT) scanning, and the NIH Opiate Total Synthesis is employed to provide previously inaccessible unnatural enantiomers of opiates and derivatives. The binding characteristics, pharmacology, immunochemistry, and the multiplicity of opioid receptors were examined, and new drugs were explored as treatment agents for cocaine abuse and for their interaction with the dopamine transporter.

Study of delta opioid receptors - The enantiomers , their benzylic epimers and the corresponding methyl ethers, of the delta opioid receptor selective racemic agonist BW373U86, were synthesized from the enantiomers of 1-allyl-trans-2,5-dimethylpiperazine. Studies with these compounds in the isolated mouse vas deferensand guinea pig ileum bioassays revealed that the delta/mu selectivity of the (+)-methyl ether of BW373U86 was about 2000 fold. This compound will be a valuable tool for further study of the delta opioid receptor structure and function, including its role in addictive diseases.

Potential agents for treatment and prevention of cocaine abuse - The cocaine receptor on the dopamine transporter is a logical target binding site for the design and synthesis of novel agents for evaluation as possible cocaine antagonists. Synthetic studies for the identification of drugs which will be useful in the treatment and prevention of cocaine abuse are continuing.

X-ray crystal structures of potent opioid receptor ligands - As a prelude to molecular modeling and other studies of the newly cloned and expressed mu, delta, and kappa opioid receptor subtypes, X-ray crystal structures were determined for etonitazene, cis-(+)-3-methylfentanyl, and etorphine, three extremely potent opioid agonists. X-ray crystal structures were also determined for diprenorphine, a potent opioid antagonist, and buprenorphine, a clinically useful mixed agonist-antagonist.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 59,502-06 LMC

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Design, Synthesis and Evaluation of Medicinal Agents and Research Tools

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A.E. Jacobson	Deputy Chief, LMC	LMC-NIDDK
Co PI:	K.C. Rice	Chief, Drug Design & Synth. Sect.	LMC-NIDDK
Others:	C. Bertha	IRTA	LMC-NIDDK
	M. Mattson	Biologist	LMC-NIDDK
	X-S. He	Special Volunteer	LMC-NIDDK

COOPERATING UNITS (if any)
LN, NIDDK (A.S. Basile, I.A. Paul); Naval Research Lab (C. George); ARC-NIDA (R.B. Rothman); Medical College of Virginia, VCU (E.L. May); University of Michigan (J. Woods); University of Mississippi Medical School (W. Woolverton).

LAB/BRANCH Laboratory of Medicinal Chemistry

SECTION Drug Design and Synthesis Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 4.0	PROFESSIONAL: 3.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ligands for the sigma receptor system were studied. Sigma receptors are non-dopaminergic, non-opioid receptors which bind antipsychotic drugs and have been implicated in neural regulation of motor behavior and modulation of transmitter release upon electrical stimulation of smooth muscle preparations:

The enantiomeric (-)- and (+)-N-alkyl (-methyl through -decyl) normetazocines (5,9alpha-dimethyl-2'-hydroxy-6,7-benzomorphans) were synthesized and their in vitro and in vivo activities determined. Increasingly bulky enantiomeric N-alkyl homologs were prepared until their interaction with the sigma-1 receptor decreased. The (+)-butyl through (+)-octyl homologs were essentially equipotent with, or more potent than, (+)-pentazocine at the sigma receptor and are thus among the most potent sigma receptor ligands known. In the levo series, all of the compounds from (-)-methyl through -octyl show antinociceptive activity, and three of them display narcotic antagonist activity. Of all of the tested compounds, only the (-)-propyl homolog is a potent narcotic antagonist. The transition, which is easily measured, from agonist ((-)-methyl) to agonist-antagonist ((-)-ethyl, -propyl, and -butyl) and back to agonist ((-)- pentyl, -hexyl, -heptyl, and -octyl) in this (-)-N-alkyl series, is not readily explainable. Conceivably, future exploration of the series at the molecular level will resolve this enigma.

An E-8-benzylidene and E-8-(3,4-dichlorobenzylidene), 7-ketone derivatives of the phenylmorphan opioid 2-methyl-5-(3-hydroxyphenyl)morphan (5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo [3.3.1]nonane) were synthesized. Radioreceptor binding studies in rat and guinea pig brain preparations revealed that the (-)-(1S,5S)-E-8-benzylidene derivative displayed an 11-fold decrease in affinity for the opioid receptor and an increase in affinity for receptors of 81-fold (low nanomolar affinity) relative to its ketone precursor. The (-)-(1S,5S)-E-8-benzylidene derivative and the (-)- (1S,5S)-E-8-(3,4-dichlorobenzylidene) are distinct from the typical sigma ligands in that they have very low affinity for either PCP sites or muscarinic receptors. The high affinity and selectivity of these novel receptor ligands suggests that they will be valuable for the elucidation of the functional roles of these receptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 59602-20 LMC

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Interferon Induction and Action. The Antiviral Action of Nucleoside Analogues

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. F. Torrence	Section Chief	LMC/NIDDK
Others:	S. Khamnei	IRTA	LMC/NIDDK
	W. Xiao	Visiting Fellow	LMC/NIDDK
	G. Li	Visiting Fellow	LMC/NIDDK

COOPERATING UNITS (if any)
Dr. R. Silverman, Cancer Biology, Cleveland Clinic Foundation, Cleveland, Ohio

LAB/BRANCH Laboratory of Medicinal Chemistry

SECTION Biomedical Chemistry

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 3.9	PROFESSIONAL: 3.9	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Interferon-induced enzyme activities such as the 2-5A synthetase, the 2',5'-phosphodiesterase, and the 2-5A-dependent ribonuclease are studied with the goal of understanding their role in the action of interferon, the induction of interferon by dsRNA, and the control of cell growth and differentiation. Analogues of a mediator of interferon action are synthesized in order to define the relationship between oligonucleotide structure and binding to and activation of the 2-5A-dependent endonuclease. The eventual goal is to understand the biological role of the 2-5A system and to explore the potential of exploitation of this system in chemotherapy. Finally, a number of new approaches to pharmacologically active nucleoside analogues are pursued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 59-701-21LMC

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Reactions and Immunochemistry of Carbohydrates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Cornelis P. J. Glaudemans	Section Chief, SOC	LMC/NIDDK
Others:	R. S. Arrepalli, E. M. Nashed	Visiting Scientists	LMC/NIDDK
	P. Kovac, E. Petrakova	Res. Chemist; Visiting Assoc.	LMC/NIDDK
	L. Mulard, L. Ping-Shen	Visiting Fellows	LMC/NIDDK
	C. Miller, M. Gotoh, Y. Ogawa	Visiting Fellow, Spec. Volunteers	LDMI, NICHD

COOPERATING UNITS (if any)

J. B. Robbins, NICHD
R. Schneerson, NICHD

LAB/BRANCH Laboratory of Medicinal Chemistry

SECTION Section on Carbohydrates

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 7.0

PROFESSIONAL: 7.0

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Section works on the interaction of (complex) carbohydrate immuno-determinants with monoclonal antibodies (MAbs). The elucidation of this interaction in great molecular detail pertains to all ligand-protein interactions. We are executing:

1. Physico-chemical studies on antibody/antigen systems (Sh. dys. type 1, V. cholera).
2. The synthesis of ligands for affinity studies.
3. The study of immunodeterminants of bacteria causing significant diseases on a global scale (Sh. dysenteriae type 1, V. cholera), so as to evaluate procedures for vaccine development.
4. The amino acid sequencing of immunoglobulins and studies on their tertiary structures.

We are continuing to determine the specific interaction between microbial polysaccharides, such as dextran and a number of monoclonal antibodies, Vibrio cholera and its monoclonal antibodies, and have prepared ligands to probe the fundamental nature of these antibody-antigen association.

We have prepared additional complex fragments of the capsular polysaccharide of Shigella dysenteriae type 1 by sophisticated syntheses. These include deoxy and deoxyfluoro derivatives of the determinant. Studies are continuing on the binding area of a monoclonal antibodies towards this disease-causing micro-organism. The amino acid sequence of some of these antibodies will be determined by cDNA approaches. Three dimensional structures will be evaluated to delineate actual molecular forces involved in the binding.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 59801-03 LMC

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Evaluation of Potential Cocaine Antagonists

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.R. Glowa	Expert, Behavioral Pharmacol. Unit	LMC/NIDDK
Others:	Frank Wojnicki	IRTA	LMC/NIDDK
	Nolana Kabwit	summer IRTA	LMC/NIDDK
	Gregory Charney	Howard Hughes student	LMC/NIDDK

COOPERATING UNITS (If any)

LAB/BRANCH Laboratory of Medicinal Chemistry

SECTION Drug Design and Synthesis Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary mission of the Behavioral Pharmacology Unit is to assess the behavioral effects of pharmacological agents, designed by the LMC and other sources, for their efficacies to modify the abuse potential of cocaine. The usual means by which this mission is accomplished is the direct assessment of these agents on drug-seeking behavior of monkeys.

A) The primary current effort of the lab is to characterize the effects of GBR 12909HCl and GBR 12935HCl, using cocaine self-administration in monkeys. GBR12909 and GBR12935 decrease cocaine-seeking behavior (up to 100%) at doses that have little or no effect on food-seeking behavior. These effects are further explored by 1) altering the maintenance dose of cocaine while assessing the effects of GBR 12909, 2) comparing these effects to other related agents having high affinity for the DA re-uptake site, including LR-1111, DM-69, DM-58, and DM-77, and 3) comparing these effects to other non-chemically related agents having affinity for the DA re-uptake site, including pemoline, buproprion and mazindol. The results show that drug-seeking behavior can be selectively attenuated by high-affinity dopamine reuptake inhibitors, suggesting that such agents may be useful in treating cocaine abuse. However, the results also suggest that other factors (e.g. lipophilicity, bioavailability, etc.) may be important in determining the behavioral effects of these agents.

B) A second line of studies furthers the association between stress and drug abuse using genetic rodent models. For example, different rat strains can exhibit large differences in hypothalamic pituitary-adrenal activity that have been used to determine the role of the neuroendocrine system in susceptibility to autoimmune disease, drug abuse and other behavioral end points. To further characterize potential behavioral correlates of these differences, the amplitude of the acoustic (ASR) and tactile (TSR) startle response and the corticosterone response to acoustic startle stimuli were compared between two histocompatible strains, Lewis (LEW/N) and Fischer (F344/N) rats, as well as outbred Harlan Sprague-Dawley (SD) rats. These findings suggested an inverse relationship between the amplitude of the ASR and hypothalamic-pituitary-adrenal activation across strains. This relationship was further supported by a high negative correlation between corticosterone level and ASR amplitude within the F344/N group.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 59802-02 LMC

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Elucidation of the Structure and Function of Sigma Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. D. Bowen	Research Pharmacologist, Receptor Biochemistry & Pharmacology Unit	LMC/NIDDK
Others:	B.J. Vilner	Visiting Scientist	LMC/NIDDK
	C. Torrence-Campbell	IRTA	LMC/NIDDK
	W. Williams, D. Thomassen	Biologist	LMC/NIDDK
	R. Goree, H. Abdul-Lateef	MARC student, Spec. Volun.	LMC/NIDDK

COOPERATING UNITS (if any)
Research Triangle Institute (F.I. Carroll); U. California, Irvine (R. Matsumoto); George Washington University Medical Center (C.S. John); Brown University (J.M. Walker); NIMH (K.-S. Lee); U. Arizona (F. Porreca).

LAB/BRANCH Laboratory of Medicinal Chemistry

SECTION Drug Design and Synthesis

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 5.5
PROFESSIONAL: 3
OTHER: 2.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Sigma receptors are saturable, high affinity binding sites for several important classes of psychotropic drugs including typical antipsychotic, antidepressant, anticonvulsant, and psychotomimetic compounds. They are likely to contribute to the beneficial and/or side-effect profile of these compounds. Our laboratory has investigated several aspects of the molecular and cellular pharmacology of sigma receptors.

Cytotoxic effects of sigma ligands on primary culture of central and peripheral nervous system: Sgma ligands produced alterations in the morphology of cells from cortex, cerebellum, thalamus, spinal cord, and superior cervical ganglion. Initial effects involved alterations of processes, followed by cell rounding or extreme swelling, and cell death. Neurons appeared to be more sensitive than non-neuronal cells which were present in the cultures. In cerebellum, BD737, reduced haloperidol, and fluphenazine produced initial effects at 10 micromol. after 7 days and at only 3 micromol. after 21 days. Several aryl ethylenediamines killed spinal cord neurons after 15 days at 3 micromol. These results have important implications for neuroleptic-induced tardive dyskinesia.

Characterization of sigma receptors in clonal cell lines: Thirteen tumor-derived cell lines from various tissues were examined for the presence and density of sigma-1 and sigma-2 receptors. These included human MCF-7 breast adenocarcinoma, T47D breast ductal carcinoma, NCI-727 lung carcinoid, A375 melanoma, U-138MG glioblastoma, SK-N-SH neuroblastoma, ThP-1 leukemia, and LNCaP prostate. All cell lines tested were found to express very high densities of sigma receptors, suggesting a role in tumor biology.

Characterization of novel ligands for sigma receptors: In an attempt to develop new sigma receptor radioligands for biochemical studies and in vivo SPECT imaging, a series of novel iodo-substituted benzamides was investigated for affinity at sigma-1 and sigma-2 receptors. Two compounds, N-[2-(piperidinylamino)ethyl]-4-iodobenzamide (IPAB) and (N-benzylpiperidin-4-yl)-4-iodobenzamide (4-IBP), were of particular interest. IPAB had high affinity and selectivity for sigma-1 receptors, whereas 4-IBP exhibited high affinity for both sigma-1 and sigma-2 receptors.

In vivo imaging of tumors using sigma receptors as targets for imaging agents: The high density of sigma sites in tumor cell lines has suggested that sigma receptor-targeted radioligands could be used to image tumors in vivo utilizing PET or SPECT technology. Using [131I]PAB, scintigraphic images of implanted melanoma and NCI-H1299 large cell lung carcinoma were produced in nude mice. Also, 4-[125I]BP was shown to bind with high affinity to MCF-7 breast tumor cells. Thus, these compounds may have tumor diagnostic potential.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69000-29 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diabetes Mellitus and Other Chronic Diseases in the Gila River Indian Community

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.C. Knowler	Chief	DAES, NIDDK
Others:	P.H. Bennett	Chief	PECRB, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	R.L. Hanson	Senior Staff Fellow	DAES, NIDDK
	K.M.V. Narayan	Visiting Scientist	DAES, NIDDK
	M. de Courten	Visiting Fellow	DAES, NIDDK
	R. Fernandes	Visiting Fellow	DAES, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; National Institute of Arthritis and Musculoskeletal and Skin Diseases; Ariz. State U.; Cleveland Clinic, Phoenix, AZ; University of Pittsburgh.

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to identify the determinants of non-insulin-dependent diabetes (NIDDM), various types of arthritis, and gallbladder disease, and elucidate the natural history of the diseases. Genetic and environmental risk factors for NIDDM have been studied in the Pima Indians. The residents of the study area, approximately 5000 people, have participated in a longitudinal population study since 1965, allowing observations of the natural history of diabetes mellitus. Risk factors for obesity, hypertension, and cholelithiasis are also studied, along with the relationships of these diseases to diabetes and their effects on mortality rates. The genetics of diabetes is studied by means of family studies and relationships of genetic markers to disease. The roles of obesity, serum insulin concentrations, impaired glucose tolerance, occupational and leisure-time physical activity and diabetes in relatives are assessed.

The prevalence of diabetes in offspring or siblings of diabetic subjects was higher if the index case was less rather than more obese. This may indicate that a greater "load" of familial determinants of diabetes is present in leaner diabetic persons and should be taken into account in familial and genetic investigations of NIDDM.

NIDDM is highly prevalent in Pima Indians; the disorder is familial, but its prevalence has increased in recent years. Segregation analysis was conducted to determine whether, accounting for the cohort effect, familial aggregation of NIDDM in this population could reflect the action of a single major gene. The findings are consistent with the hypothesis that a major gene influences the risk for NIDDM in Pima Indians by affecting age at onset. The expression of this gene may depend on environmental factors that have become more prevalent in recent birth cohorts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 LK 69001-25 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Complications and Outcome of Diabetic and Prediabetic Pregnancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.J. Pettitt	Assistant Chief	DAES, NIDDK
Others:	P.H. Bennett	Chief	PECRB, NIDDK
	W.C. Knowler	Chief	DAES, NIDDK
	R.L. Hanson	Epidemiology Staff Fellow	DAES, NIDDK
	K.M.V. Narayan	Visiting Scientist	DAES, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; Biostatistics and Data Management Section, PECRB

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	0.7	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purposes of this project are to determine the effects of abnormal glucose tolerance on outcome of the pregnancy, to determine long-term prognosis for the women and their offspring, and to identify diabetes and impaired glucose tolerance during pregnancy in women in the Gila River Indian Community. By means of a glucose tolerance test as well as chart review, the diabetes status of every woman is determined at two-yearly intervals and during the third trimester of each pregnancy. As third trimester glocysylated hemoglobin did not predict future diabetes while third trimester 2-hour glucose, obesity, age and particy each did, we studied the correlation between glycosylated hemoglobin and plasma glucose in pregnant and nonpregnant women and evaluated the chnages in glycosylated hemoglobin which occurred with pregnancy. Controlled for age, BMI, and gravidity, glycosylated hemoglobin and 2-hour plasma glucose were more highly correlated in 2124 nonpregnant ($r=0.86$) than in 1207 pregnant ($r=0.30$) women. In 369 normoglycemic women examined both when pregnant and when not pregnant, the glycosylated hemoglobin was lower during pregnancy by approximately 0.45% ($p<0.05$). Controlled for age, BMI, and gravidity, the 2-hour plasma glucose predicted future diabetes in both nonpregnant and pregnant women, but glycosylated hemoglobin did so only in nonpregnant women.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69006-24 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gila River Indian Community Autopsy and Mortality Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	PECRB, NIDDK
Others:	W.C. Knowler	Chief	DAES, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	M.L. Sievers	Guest Researcher	DAES, NIDDK
	R.L. Hanson	Epidemiology Staff Fellow	DAES, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The causes of death and postmortem characteristics of Pima Indians of the Gila River Indian Community are investigated so that findings in subjects with and without diabetes mellitus can be correlated with studies in living subjects. Medical records are reviewed for the determination of cause of death and for the occurrence of certain serious diseases or complications of diabetes.

The purpose of the study is to relate the outcome and cause of death to events or risk factors measured in life among Pima Indian residents of the Gila River Indian Community, particularly in relation to diabetes, cardiovascular diseases and gallbladder disease. Death Certificates are obtained on all members of the Gila River Indian Community In addition, post mortem examinations and all available medical records pertaining to the subjects are obtained to ascertain conditions present at the time of death and ascertain cause of death as precisely as possible. These records are reviewed in a standardized way for evidence of the complications of diabetes, vascular disease, neoplasms and other conditions. The records are used to determine the causes of death and incidence of complications associated with diabetes and other conditions identified initially during life by the longitudinal epidemiologic studies in the population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 69009-25 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Natural History of Arthritis and Rheumatism in the Gila River Indian Community

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	PECRB, NIDDK
Others:	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	W.C. Knowler	Chief	DAES, NIDDK
	S.P. Heyse	Chief	OPECA, NIAMS
	R. Fernandez	Visiting Fellow.	DAES, NIDDK
	R. Hirsh	IRTA	OPECA, NIAMS

COOPERATING UNITS *(if any)*

Indian Health Service, Arizona State University; NIAMS

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.3	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development and progression of osteoarthrosis, rheumatoid arthritis and ankylosing spondylitis are being determined by means of clinical, radiographic and serological examinations carried out prospectively at two-yearly intervals among adults of the Gila River Indian Community (Pima Indians) in Arizona, in conjunction with epidemiological studies of diabetes in the same community. The purpose of this investigation is to ascertain the determinants of these diseases in the population, and to identify factors which predispose to or alter the natural history of progression of the disease. Host factors such as age, sex, and various gene markers including HLA and Gm, together with various potential environmental determinants, such as obesity and evidence of exposure to infectious agents, are being investigated prospectively to determine their relationship to the development of these diseases. Longitudinal data have now been collected for 25 years and represent a unique data set for epidemiological studies of arthritis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69015-12 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cross-Sectional and Longitudinal Study of "pre-diabetes" in the Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS, NIDDK
Others:	D. Mott	Supervisory Res. Chem.	CDNS, NIDDK
	R. Pratley	Senior Staff Fellow	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.05	1.35	.70

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Pima Indians of Arizona have the highest reported prevalence and incidence of non-insulin dependent diabetes mellitus (NIDDM) of any population in the world. Beginning in 1982, our section has studied a subset of this population to determine the etiologic factors that predispose non-diabetic individuals to develop the disease. Subjects are admitted yearly to the clinical research ward to undergo body composition analysis, an oral glucose tolerance test, an intravenous glucose tolerance tests, a standard mixed meal test, and a two-step hyperinsulinemic, euglycemic clamp to measure insulin action in vivo. Approximately 400 individuals have entered into the study and approximately 50 subjects have developed NIDDM. Subjects with NIDDM are characterized by; 1) obesity, 2) central obesity, 3) insulin resistance, 4) insulin secretory dysfunction, and 5) overproduction of glucose by the liver. Obesity, central obesity, fasting hyperinsulinemia, and insulin resistance are each major risk factors for the developement of NIDDM. The acute insulin response to a intravenous glucose bolus is an additional, but weaker predictor. The rate of post-absorptive of hepatic glucose production is not predictive of the disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69020-10 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Insulin Resistance and the Regulation of Muscle Glycogen Synthase Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS, NIDDK
Others:	D. Mott	Supervisory Res. Chem.	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; Department of Pharmacology, Duke University Medical Center, Durham, NC (Richard S. Surwit)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.1	1.1	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are currently characterizing the abnormalities for regulation of human muscle glycogen synthesis in insulin-resistant subjects. In insulin-resistant subjects, fasting glycogen synthase phosphatase (PP-1) activities are reduced and fail to show the peak insulin stimulation observed for insulin-sensitive subjects at 10-20 minutes. All insulin resistant subjects tested showed Mn activation of trypsin treated PP-1 in the absence of azide. 6 of 10 insulin sensitive subjects, however, required azide in order to see Mn activation of PP-1. The azide appears to reverse the effects of an inhibitor of Mn activation which as been localized in the glycogen-microsomal (GM) subcellular fraction of Mn-resistant (insulin sensitive) subjects. These results suggest that an azide sensitive structure in the GM fraction of muscle is responsible for the abnormal PP-1 activity in insulin resistant subjects. Phosphorylation of the G-subunit bound to the PP-1 catalytic subunit in the GM fraction regulates PP-1 activity. Western blots of muscle extracts indicate that the G-subunit has increased immunoreactivity in insulin resistant subjects. A human homolog of rat liver PP-1 gamma cDNA was isolated from human skeletal muscle. This observation indicates that at least three PP-1 isoforms may be involved in regulation of glycogen synthase. Insulin resistant subjects have increased binding of their PP-1 gamma isoform to the GM fraction. Although PP-1 alpha and beta also bind to the GM fraction, no differences were observed between insulin sensitive and resistant subjects. Taken together these results suggest that abnormal PP-1 activity in insulin resistant subjects may directly involve interaction between the G-subunit and PP-1 gamma. Studies of inbred mouse strains demonstrate that a diabetes susceptibility locus close to the glycogen synthase gene on mouse chromosome 7 is present in a strain of mice which have abnormal glycogen synthase activity. These results support the hypothesis that a defect in the glycogen synthase gene can contribute to the development of diabetes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69021-13 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Energy Expenditure in Pima Indians: Risk Factors for Body Weight Gain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Ravussin	Visiting Scientist	CDNS, NIDDK
Others:	R. Norman	Senior Staff Fellow	CDNS, NIDDK
	S. Snitker	Visiting Associate	CDNS, NIDDK
	P.A. Tataranni	Special Volunteer	CDNS, NIDDK
	C. Bogardus	Chief	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; Brighman Young University (M. Rowe); Rockefeller University (R. Liebel); Arizona State University (W. Willis)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4	2.7	1.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Since obesity represents a major risk factor for insulin resistance in the development of NIDDM, over the past 10 years we have focused our efforts in searching for the possible causes of obesity in the Pima Indian population. We have identified 4 familial metabolic parameters predicting body weight gain; a) a low metabolic rate, b) a high respiratory quotient, c) insulin sensitivity and d) a low spontaneous physical activity. We are presently investigating the underlying mechanisms of the variability of these 4 metabolic parameters. Recent data suggests that Pima Indians, have not only low levels of physical activity in adulthood, but already at the age of 5. Presently, we are conducting prospective studies of the impact of the level of physical activity assessed by doubly-labeled water on subsequent weight change. We are also pursuing studies designed to better assess the role of sympathetic nervous activity in carbohydrate metabolism, temperature regulation, substrate oxidation, and cardiovascular responses in this population. Since a low fat oxidation is a risk factor for body weight gain, we have recently demonstrated that skeletal muscle lipoprotein lipase activity and muscle fiber types account for part of this interindividual variability in macronutrient partitioning. In our search for linkages of obesity phenotypes and sub-phenotypes to candidate genes we have recently found that the tumor necrosis factor α gene on chromosome 6 is linked to body fatness. However, this linkage is not related to any variant in the 3 exons of the genes. We are performing more obesity linkage studies with candidate genes and homologous regions of rodent obesity genes. Recent studies of mitochondrial DNA have provided an association between a variant in the sub-unit 1 of the NADH dehydrogenase gene and resting metabolic rate. However, this variant is not associated with differences in in vitro mitochondrial respiration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69024-08 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

WHO Collaborating Center for Epidemiologic and Clinical Investigations in Diabetes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	PECR, NIDDK
Others:	W.C. Knowler	Chief	DAES, NIDDK
	C. Bogardus	Chief	CDNS, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK

COOPERATING UNITS *(if any)*

World Health Organization, Non-Communicable Diseases Program, Geneva, Switzerland, (Foreign), World Health Organization Collaborating Centers for Diabetes (Foreign), China-Japanese Friendship Hospital, Beijing, China (Pan Xiaoren) (Foreign)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.1	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Phoenix Epidemiology and Clinical Research Branch was designated as the WHO Collaborating Center for Design, Methodology and Analysis of Epidemiological and Clinical Investigations in Diabetes in 1986. The purposes of the Center are to collaborate with the World Health Organization in the implementation of the WHO/IDF action program to provide advice, consultation and collaboration with other investigators in the design, methodology and analysis of epidemiology and clinical diabetes (NIDDM) and its complications. The center assists in the development and application of standardized methods for epidemiological and clinical investigations and data analysis relating to the etiology and pathogenesis of non-insulin-dependent diabetes (NIDDM) and its complications. The center will collaborate with those interested in applying such techniques elsewhere. The Center will advise and help in the design of new studies, including on site assistance when necessary. The Center serves to train investigators from many parts of the world in diabetes epidemiology and clinical research.

The Center participates in the WHO Multicenter Study of Vascular Disease in Diabetes, which is examining the mortality and incidence of vascular complications of diabetes among different ethnic groups in different countries.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69025-08 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Treatment of Impaired Glucose Tolerance in Malmöhus County Sweden

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: W.C. Knowler Chief DAES, NIDDK

COOPERATING UNITS *(if any)*

Lund University, Lund, Sweden (Foreign)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The prognosis and effect of treatment of impaired glucose tolerance (IGT) are studied in residents of Malmöhus County, Sweden. Mortality rates are determined in over 2000 persons who had glucose tolerance tests in the 1960s, and results are related to glucose tolerance and other factors at baseline. Some of these subjects participated in a randomized treatment study of IGT. New studies in population screening and treatment of IGT have been planned.

This project is terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69028-06 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetics of Non-Insulin-Dependent Diabetes Mellitus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.C. Knowler	Chief	DAES, NIDDK
Others:	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	P.H. Bennett	Chief	PECRB, NIDDK
	C. Bogardus	Chief	CDNS, NIDDK
	M. Prochazka	Senior Staff Fellow	CDNS, NIDDK
	R.L. Hanson	Senior Staff Fellow	DAES, NIDDK

COOPERATING UNITS *(if any)*

NIAAA; Bowman-Gray Medical School, Winston-Salem, NC; Indiana University, Indianapolis IN; University of Chicago, Chicago IL; Arizona State University, Tempe, AZ.

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Non-insulin dependent diabetes mellitus is a common chronic disease that develops in most populations in late middle age. The Pima Indians of Arizona have the highest reported prevalence of this disease in the world and in contrast to many populations the disease often presents at an earlier age. As a result of long-term epidemiological studies in the total population, the familial nature of the disease has been well documented, and segregation analyses suggest the possibility of inheritance by a single additive major gene.

This project will search for genetic determinants of NIDDM using the techniques of genetic linkage analysis with genetic markers to identify the chromosomal location of inherited determinants of NIDDM in the Pima Indian population. A number of informative pedigrees have been identified and lymphoblast cell lines from informative members of these pedigrees established. DNA from these lymphoblasts is isolated and polymorphic probes applied to search for evidence of linkage of these markers and NIDDM. Probes with established chromosomal locations will be used to screen the genome to detect genetic linkage with NIDDM as described in Project# Z01 DK 69045-01 (Molecular genetic analysis of a chromosome 4 region harboring agent controlling insulin action).

The power to detect linkage was estimated under several sets of assumptions about mode of inheritance of diabetes and informativeness and location of DNA markers. With a large number of sib-pairs and highly polymorphic markers, the power to detect a major diabetes-susceptibility gene in the Pima is high.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69030-06 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Contribution of Protein Tyrosine Phosphatase to Insulin Resistance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS, NIDDK
Others:	J. Rowles	IRTA	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.05	1.05	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously identified a novel PTPase in human skeletal muscle by PCR and reported the cloning of the full-length cDNA. This PTPase is called PTP-PEST due to the presence of four regions rich in pro, glu/asp and ser/thr. To assess if the PEST sequences serve any regulatory role, the full length protein (PTP-PEST) and a truncated version that contains the entire catalytic domain but lacks all the PEST sequences (PTP-PESTdelta) have been expressed in Sf9 cells using a recombinant baculovirus. Each of these expressed proteins have been purified and found to have specific activities and affinities for an insulin receptor peptide substrate that do not vary by more than 2-fold. To determine if these PTPs may be involved in insulin signalling, they were coexpressed with a recombinant baculovirus that produces a soluble form of the insulin receptor tyrosine kinase (SIR), (kindly provided by Melanie Cobb, UTSWMC). Co-expression of PTP-PEST or PTP-PESTdelta with the SIR caused a reduction in the P-tyr content of SIR; this decrease correlated with a decrease (40-600) in the tyrosine kinase activity of the SIR measured against an artificial substrate, PEY. Interestingly, there was also a decrease (40-60%) in the PTPase activities of both PTP-PEST and PTP-PESTdelta when coexpressed with the SIR. To determine if this effect of the SIR was a direct one, extracts of Sf9 cells were blotted with P-tyr antibodies. No prominent bands corresponding to the sizes of the PTPs were detected using this method. In addition, preliminary results of phosphoamino acid analysis of PTP-PESTs from $^{32}P_i$ labeled Sf9 cells indicate only ser/thr phosphorylation. In support of these in vivo data, the in vitro stoichiometry of phosphorylation of the PTPs by SIR was negligible; 0.08 mol/mol for PTP-PEST and 0.01 mol/mol for PTP-PESTdelta. We have very recently obtained an antiserum against a PTP-PEST peptide that recognizes both forms of the PTPs by Western blot, and early indications are that it will also be very useful for immunoprecipitation. This antibody should allow us to determine protein levels and possibly enzyme activities of PTP-PEST in skeletal muscle from insulin sensitive and resistant Pimas.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69036-05 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Epidemiology of Complications of Non-Insulin-Dependent Diabetes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.C. Knowler	Chief	DAES, NIDDK
Others:	P.H. Bennett	Chief	PECRB, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	R.L. Hanson	Senior Staff Fellow	DAES, NIDDK
	K.M.V. Narayan	Visiting Scientist	DAES, NIDDK
	M. de Courten	Visiting Fellow	DAES, NIDDK
	R. Fernandes	Visiting Fellow	DAES, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; Cleveland Clinic Foundation, Phoenix, AZ.

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.6	1.9	0.7

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of the project is to determine the incidence rates, rates of progression, and risk factors for the chronic complications of NIDDM. The study is conducted in the Pima Indians of the Gila River Indian Community, who have participated in a longitudinal epidemiologic study since 1965 (see project Z01 DK 69000).

Risk factors for the major complications of diabetes, retinopathy, nephropathy, coronary artery disease, and peripheral vascular disease are determined by longitudinal followup of diabetic subjects. Methods of ascertainment of these complications include fundus photography, measurement of urine albumin and serum creatinine concentrations, electrocardiography, and documentation of lower extremity amputations.

The incidence of elevated urinary albumin excretion in Pima Indians with NIDDM is at least as high as that reported in IDDM, and its major risk factors (retinopathy, type of diabetes treatment, longer duration of diabetes, lower body mass index, and higher values of mean arterial pressure, glycated hemoglobin, and fasting and two-hour post-load plasma glucose concentrations) are the same as those shown previously to predict the development of more advanced renal disease in this population.

Diabetic subjects with peripheral neuropathy had slightly higher age-sex adjusted mortality rates from all causes and a 2.2-fold increase in mortality rates due to renal causes compared with diabetic subjects without neuropathy. These findings are consistent with the hypothesis that peripheral neuropathy is associated with autonomic neuropathy and impaired renal autoregulation, which may predispose to glomerular injury.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69037-05 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Kidney Function in Non-Insulin-Dependent Diabetes Mellitus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	PECRB, NIDDK
Others:	W.C. Knowler	Chief	DAES, NIDDK

COOPERATING UNITS *(if any)*

Cleveland Clinic Foundation; Stanford University; Emory University; Chronic Renal Diseases Program, NIDDK; Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.2	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The functional characteristics of the renal glomerulus have been investigated in Pima Indians of the Gila River Indian Community to identify the underlying pathogenetic mechanisms involved in the initiation and progression of renal disease in non-insulin-dependent diabetes mellitus (NIDDM).

The Pima Indian population has a high incidence of NIDDM and diabetic nephropathy. Six groups of subjects were studied: subjects with normal glucose tolerance, individuals with impaired glucose tolerance (IGT), those with newly diagnosed diabetes (<3 years duration NIDDM), and diabetic subjects (≥ 5 years duration NIDDM) with evidence of (a.) mildly abnormal albumin excretion (b.) severe abnormalities of albumin excretion (c.) normal albumin excretion. Measurements of renal and glomerular capillary wall function including glomerular filtration rate (GFR), renal plasma flow (RFP), dextran sieving coefficients, and albuminuria were performed and correlated. Markers and/or predictors of progression as well as the mechanisms of initiation and progression of diabetic renal disease were sought. Follow-up over a 48 month period has been completed.

The project is now terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69039-05 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Dietary Survey of the Pima Indians of the Gila River Indian Community

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.C. Knowler	Chief	DAES, NIDDK
Others:	P.H. Bennett	Chief	PECRB, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	V. Narayan	Visiting Scientist	DAES, NIDDK

COOPERATING UNITS *(if any)*

Cleveland Clinic Foundation, Phoenix, Arizona.

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An age-sex-stratified sample of 600 residents of the Gila River Indian Community, ages 18-75 years, was recruited for a dietary survey. Dietary intake was estimated by the dietary history method to obtain quantitative food frequency information. Reported energy intake was higher in men and negatively related to age; thus, relationships with weight were analyzed by multiple regression controlling for sex and age. Energy intake in kilocalories (kcal) was positively associated with body weight or body mass index, adjusted for age and sex. Body weight was associated with absolute intake of the major dietary components, carbohydrate, fat, and protein, but not with any of these components expressed per 1000 kcal, suggesting that total energy intake, rather than proportions of specific components, was the variable having the strongest association with body weight. Alcohol consumption (in any amount) was higher in men and inversely associated with age and weight. Neither energy intake nor specific components were significantly associated with diabetes, after adjustment for age and sex, nor did diabetes affect the relationship between energy and weight, in a subset of 307 subjects (153 diabetics) examined for diabetes within one year of the diet history. The acceptance of obesity in this population may reduce the under-reporting of energy intake which has been postulated in other studies. Body weight in Pima Indians is weakly but positively associated with increased caloric intake.

In contrast to the findings from many other studies, reported calcium intake was positively related to blood pressure. Such associations are not uniform between populations, suggesting the importance of unmeasured confounding variables.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69040-05 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sodium-Lithium Countertransport and Blood Pressure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.C. Knowler	Chief	DAES, NIDDK
Others:	D.J. Pettitt	Assistant Chief	DAES, NIDDK

COOPERATING UNITS *(if any)*

University of Pittsburgh; University of Southern California

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1 0.1	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Red blood cell sodium lithium countertransport is a genetic marker of hypertension in several ethnic groups. It reflects the sodium-hydrogen antiport activity in renal tubules. Several recent studies showed an association between sodium-lithium countertransport and predisposition to diabetic nephropathy. We are studying the relationships between sodium-lithium countertransport, nephropathy, and blood pressure in a sample of 200 Pima Indians.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69045-03 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of the Intestinal Fatty Acid Binding Protein in Insulin Resistance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS, NIDDK
Others:	L. Baier	IRTA	CDNS, NIDDK
	M. Prochazka	Senior Staff Fellow	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; Department of Biochemistry, Albert Einstein Collge of Medicine, Bronx NY (J. Sacchettini).

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our previous linkage study indicated that a gene on chromosome 4q contributes to insulin action in Pimas. A candidate gene in this region is FABP2 which encodes the intestinal fatty acid binding protein (IFABP). To determine whether FABP2 has a role in insulin action, we analyzed the FABP2 coding sequences and detected a single base substitution in exon II that would result in an alanine (Ala) to threonine (Thr) substitution at position 54 of the protein. Pimas with a Thr-encoding FABP2 allele (frequency 0.3) were found to have increased insulin resistance and increased fat oxidation rates as compared to Pimas with an Ala-encoding FABP2 allele (frequency 0.7). To analyze these IFABP proteins biochemically for functional variation that could result in the clinical variation seen in the Pimas, we isolated the cDNA for the Ala form of IFABP and produced the cDNA for the Thr form by site-directed mutagenesis. Both cDNAs were expressed in E. coli and their products purified to homogeneity. Three-dimensional structural variation between the two proteins is currently being analyzed by X-ray crystallography. Functional variation was observed in the two recombinant proteins' affinity for long chain fatty acids, where the Thr form binds twice as tightly as the Ala form in titration microcalorimetry studies. The cDNA for each protein was also permenantly transfected into enterocyte-like tissue culture cells. Upon exposure to radiolabeled fatty acids, cells expressing the Thr protein processed fatty acids twice as quickly as cells expressing the Ala protein. Since fatty acid bound to IFABP interacts with long chain acyl CoA synthetase to form of acyl CoA, we will analyze the interaction of both forms of IFABP with acyl CoA synthetase. To this end, we have isolated the human intestinal acyl CoA synthetase cDNA and expressed this protein in yeast.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69046-03 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of the Glycogen Synthase Gene in Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Prochazka	Senior Staff Fellow	CDNS, NIDDK
Others:	C. Bogardus	Chief	CDNS, NIDDK
	M. Majer	Special Volunteer	CDNS, NIDDK
	D. Mott	Supervisory Res. Chemist	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	0.3	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have undertaken a "candidate" gene approach to search for gene(s) predisposing for insulin resistance and NIDDM in the Pima Indians. During a screening (by sib-pair linkage analysis) of polymorphic markers at selected candidates, we have found evidence for a possible linkage of the gene for skeletal muscle glycogen synthase (GYS) on chromosome 19q with fasting insulin and with measures of insulin action in-vivo. Detailed comparative analysis of GYS mRNA and protein levels in skeletal muscle of insulin sensitive and resistant Pimas revealed a 30% reduction of the protein in the latter group. In contrast, no differences in mRNA could be detected between the two groups. Because this phenomenon could be caused by a decrease in the stability of the protein due to amino acid substitution(s), analyses comparing GYS coding sequences and enzymatic activity between insulin sensitive and resistant subjects are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69049-03 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

SSCP Analysis of PP-1 Alpha and Gamma in Relationship to Insulin Resistance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Thompson	Senior Staff Fellow	CDNS, NIDDK
Others:	D. Mott	Supervisory Res. Chem.	CDNS, NIDDK
	S. Norman	IRTA	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

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Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.30	.20	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous studies on the type-1 serine/threonine protein phosphatase (PP-1) activity in skeletal muscle from fasted subjects indicated increased amounts of activity in insulin-sensitive individuals, but increased protein amounts in insulin-resistant subjects. New isoforms of PP-1 have been cloned from muscle cDNA suggesting that PP-1 activity may be a composite of several proteins. Mutations in the coding regions of the skeletal muscle PP-1 isoforms could explain the activity and enzyme content differences. Currently, the genes for the skeletal muscle protein phosphatase isoforms are being characterized. The genomic clones for these genes are being analyzed and suitable PCR primer pairs chosen from the non-coding regions that will allow amplification of PP-1 coding regions. The coding regions of these genes are being examined in insulin-sensitive and -resistant persons for single stranded conformational polymorphisms (SSCP). SSCPs that relate to amino acid changes will be further tested for a relationship to insulin resistance and type II diabetes. At this time SSCP analysis has been completed on 40 subjects for 6 exons from PP-1 gamma-1. This covers 95% of the coding sequence. No sequence abnormalities were observed which were uniquely associated with insulin resistance. This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 69050-03 PECR

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Aspects of the Acute Insulin Response in Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Thompson	Senior Staff Fellow	CDNS, NIDDK
Others:	C. Bogardus	Chief	CDNS, NIDDK

COOPERATING UNITS *(if any)*
Indian Health Service; Arizona State University (R. Janssen)

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION
NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.15	.15	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous observations of variation associated with the acute insulin response suggested that his phenotype was controlled by on or more genes. Glucokinase, which has been linked to the MODY form of diabetes, has also been proposed as a intracellular glucose sensor appeared to be a likely candidate gene. Similarly, glut-2 the major glucose transporter of the pancreas could also be responsible for the variation observed in the acute insulin response. Polymorphisms at both of these genes have been examined for an association or linkage with the acute insulin response, and analysis of 117 sibpairs suggests there is linkage between this region on chromosome 3 and the acute insulin response. A structural analysis of the coding regions for the gene encoding glut-2 revealed a single transition which predicts an amino acid substitution at position 110 of Glut2. It is currently unknown how the mutation effects glucose sensing in the Beta-cell. Additional short tandem repeat polymorphisms (STRPs) have been genotyped in the region of GLUT2. Specifically, an additional STRP within GLUT2, with higher heterozygosity, was genotyped in 1000 Pima Indians. Sibpair linkage analysis did not support our earlier observation of week linkage to the acute insulin response. While linkage to the acute insulin response was not supported, linkage (p-0.02) to maximum body mass index was observed. Two other markers flanking Glut2 also showed linkage to this trait. The marker D3S1237, proximal to Glut2, showed the tightest linkage to maximum body mass index ($p=0.0006$). Project is now part of Genetic and Physical Mapping of Phenotypic Traits in Pima Indians (Z01 DK 69057, and terminated as independent project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69051-02 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Prevention of Non-Insulin-Dependent Diabetes Mellitus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W.C. Knowler	Chief	DAES, NIDDK
Others:	K.M.V. Narayan	Visiting Scientist	DAES, NIDDK
	R.L. Hanson	Senior Staff Fellow	DAES, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	P.H. Bennett	Chief	PECRB, NIDDK

COOPERATING UNITS (if any)

Indian Health Service; Cleveland Clinic, Phoenix, AZ; University of Pittsburgh.

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	1.0	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Prevention strategies will be developed to take advantage of the metabolic and behavioral risk factors for NIDDM which have been identified in research with the Pima Indians and elsewhere. Obesity, impaired glucose tolerance, hyperinsulinemia, physical inactivity, and high fat diet have been implicated as risk factors for NIDDM. Since these factors are potentially reversible with behavioral and pharmacologic therapy, strategies for prevention will be developed and tested. A feasibility study of diet and exercise treatment is being conducted among overweight Pima Indians with normal glucose tolerance. Knowledge and experience gained from this pilot study will be used in planning and implementing the national, multicenter clinical trial in prevention of NIDDM, in which the investigators will participate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69052-02 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of SH2-Domain PTPase by Insulin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS, NIDDK
Others:	S. Sell	Special Volunteer	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; Cantenberg Center for General & Tumor Immunology, The Hebrew University Jersusalem, Israel (D. Naor)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.9	.5	.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The abnormal regulation of insulin signalling in insulin-resistant subjects is associated with inadequate suppression of soluble protein tyrosine phosphatase (PTPase) activity in skeletal muscle. Multiple PTPases are likely to be involved in the signalling cascade. SH2-Domain PTPases are a class of PTPases selected as candidates for regulating tyrosine phosphorylation following insulin stimulation since it has been reported that such PTPases associate with several receptors and the post-receptor target, IRS1. SH2-Domain PTPase expression was detected in FAO cells, a rat insulin-sensitive hepatoma cell line, and in C2C12 cells, a mouse muscle cell line, using RT-PCR and degenerate primers. Sequence analysis of the amplified products revealed a high degree of identity with the hematopoietic SH2-Domain PTPase, PTP1C. We observed evidence of alternative splicing of the PTP1C pre-mRNA in human rhabdomyosarcoma cells and in EBV-transformed lymphocytes. This putative alternatively spliced form of PTP1c will be sequenced and characterized. A related PTPase PTP1D, (SH-PTP2, SYP) has been recently found to be expressed in adult liver and skeletal muscle. PTP1D pre-mRNA is alternatively spliced in a region corresponding to the catalytic domain of the PTPase. Relative amounts of the two PTP1D isotypes will be compared in insulin resistant and sensitive subjects. Antibodies specific for this PTPase are commercially available and will be used to compare protein levels and PTPase activity. We plan to take advantage of a tumor cell line, LB T-cell lymphoma (D. Naor), to explore the role of this family of PTPases in insulin mediated signal transduction since these cells have the unique property that they require insulin for growth and they are devoid of IGF2 receptors. These features offer specificity in signalling and reduce the complexity of the signalling pathways to be studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69053-02 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Alternative Splicing by Insulin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS, NIDDK
Others:	S. Sell	Special Volunteer	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; Department of Pathology, Baylor College of Medicine, Houston, TX (T. Cooper)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.9	.5	.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Alternative splicing is a powerful regulatory mechanism for generating subtle changes in isotype distribution. The insulin receptor itself is alternatively spliced in a developmental and tissue-specific manner. A change in distribution of the two isotypes has the potential to contribute to insulin resistance since the two isotypes possess different insulin responsive properties. Differing reports on changes in the insulin receptor isotype in diabetic and control subjects prompted us to examine the isotype distribution in Pima subjects and to characterize a potential role for insulin itself in the regulation of the alternative splicing of the insulin receptor. A small and significant difference in insulin receptor isotype was detected in insulin resistant Pima subjects compared to insulin sensitive Pima subjects using RT-PCR of RNA prepared from skeletal muscle biopsies. Insulin transiently influenced the insulin receptor isotype distribution following insulin treatment of FAO cells, a rat insulin-sensitive hepatoma cell line. These insulin inducible changes in insulin receptor isotype distribution are thought to occur as a consequence of hyperinsulinemic response to insulin resistance which has some other cause(s). Changes in the isotype distribution may be a useful biochemical marker for identifying subclinically insulin resistant subjects at an increased risk for developing diabetes. We plan to compare insulin receptor isotype distributions in individuals, who are insulin sensitive but have a family history of diabetes, to determine if changes in isotype distribution are present prior to the presence of clinically detectable changes in insulin-sensitivity. These findings will be expanded to include other putative insulin responsive messenger RNAs as candidates for marker development (T. Cooper). Fractionated blood cells will be used to carry out these experiments since these cells can be easily obtained and display sensitivity toward insulin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69054-01 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Energy Expenditure and Body Composition in Patients with Spinal Cord Injuries

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Pratley	Senior Staff Fellow	CDNS, NIDDK
Others:	E. Ravussin	Visiting Scientist	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.7	.7	.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lean body mass is an important determinant of resting and 24-hour energy expenditure. Individuals who suffer spinal cord injuries experience a marked loss of muscle mass which may decrease energy expenditure and promote an increase in body fat. This project tests the hypothesis that energy expenditure is decreased in patients with spinal cord injuries as a consequence of their marked muscle atrophy. Resting metabolic rate will be measured by indirect calorimetry and body composition by dual-energy X-ray absorptiometry (DXA) in subjects with spinal cord injuries and compared to results obtained in age-matched able-bodied individuals. In addition, 24-hour energy expenditure will be measured in a respiratory chamber in a subgroup of paraplegic individuals who are fully independent in self care. A better understanding of the relationship between changes in body composition and energy expenditure in patients with spinal cord injuries may eventually suggest strategies to reduce the excess risk of diabetes and cardiovascular disease in this population. These studies also may improve our understanding of the contributions of muscle mass to energy expenditure in the general population as well as in other special populations characterized by a loss of muscle mass (such as the elderly).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69055-01 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Linkage of Markers on Chromosome 7 with NIDDM in Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Prochazka	Senior Staff Fellow	CDNS, NIDDK
Others:	D.B. Thompson	Senior Staff Fellow	CDNS, NIDDK
	C. Bogardus	Chief	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service, The Hospital for Sick Children, Toronto, Canada (S.W. Scherer, L.C. Tsui)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes & Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.6	.6	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pima Indians have the highest known prevalence of NIDDM, and previous studies indicated that genetic factors contribute to this predisposition. We have initiated a systematic search for NIDDM gene(s) by sib-pair linkage analysis of microsatellite markers covering all autosomes and the X chromosome. During the first phase of this project (aimed to type markers initially at a 20cM average distance), we have detected possible linkage of NIDDM with D7S479 on 7q21.3-q22.1. We have expanded this observation by analyses of 13 additional markers, most of them clustered within 25cM around D7S479. We have found indication of a possible linkage with NIDDM also for D7S527, D7S491, and D7S518, which are all within less than 6cM from D7S479. We conclude that our linkage data are consistent with the location of a putative NIDDM locus near D7S479. Several loci were mapped previously to this segment of 7q, and the physical position of D7S527, D7S479, D7S491, D7S518, and of the cloned genes within 7q21-q22 has already been determined by YAC contig analysis. We are considering three genes as potential candidates. These include TAC2 (encoding a precursor of several neuropeptides of the tachykinin family), GNB2 (encoding beta-2 subunit of GTP-binding regulatory proteins), and ACHE (encoding acetylcholine esterase) because they are located within physical proximity to D7S479 (approximately 3 mb), and could affect control of glucose homeostasis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 69056-01 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Linkage Disequilbrium as a Method to Map Genes for Common, Complex Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS, NIDDK
Others:	S. Sell	Special Volunteer	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; Dept. of Neurogenetics, UCSF, San Francisco, Clinical Genetics (N. Freimer), University of Leiden, The Netherlands (L. Sandkuiji)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Dibaetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.7	.5	.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Identifying regions of maximal linkage disequilbrium between a disease phenotype and a particular marker allele is a valuable means to pinpoint the chromosomal location of the responsible gene. Indeed, most of the efforts to positionally clone human disease genes have relied on the measurement of linkage disequilbrium. Markers on chromosomes 4 and 21 will be used to carry out linkage disequilibrium analysis of the Pima genome. These chromosomes were chosen for this study because they have been extensively mapped genetically and physically, and gene poor and gene rich regions have been identified and correlated to sequence composition over extensive lengths of chromosome 21. We have identifed four additional chromosomal regions as possible linkage disequilibrium candidates because they show a high degree of genetic homogeneity in the Pima population. Increased homozygosity at these four microsatellite marker loci was found in the Pima genome following a random screen with 100 markers. Linkage disequilibrium analyses will be carried out using closely spaced markers in these regions as well. The statistical anslyses of the typing data will be carried out by our collaborator. We are investigating the possibility that these regions of homogeneity might be associated with disease susceptibility in this population with a high prevalence of obesity and diabetes mellitus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69057-01 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic and Physical Mapping of Various Phenotypic Traits in Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Thompson	Senior Staff Fellow	CDNS, NIDDK
Others:	C. Bogardus	Chief	CDNS, NIDDK
	M. Prochazka	Senior Staff Fellow	CDNS, NIDDK
	V. Ossowski	Microbiologist	CDNS, NIDDK
	R. Janssen	Biologist	CDNS, NIDDK
	W. Appel	Biologist	CDNS, NIDDK
	J. Sutherland	Biologist	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service, Diabetes and Arthritis Epidemiology Section, PECR

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	0.0

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To produce a 25 cM base genetic map in Pima Indians, we have genotyped over 150 highly heterozygous short tandem repeat polymorphisms, STRPs, scattered across the genome in a data set of 1000 individuals comprising 250 nuclear families. The average heterozygosity in Pima Indians is approximately 11% lower than the reported average heterozygosity in Caucasians for these STRPs. Linkage analysis using these STRP markers has revealed regions on chromosomes 1,6, and 7 that show linkage to various aspects of carbohydrate or fat metabolism or show linkage to diabetes. We have genotyped additional markers in the regions of linkage, and have started to physical mapping on chromosome 1 with several YACs which include the marker showing linkage. In addition, a region on chromosome 20 showed strong linkage to the quantitative character, stature. Analysis of additional markers supports the original linkage observation. A candidate gene, the bone morphogenetic protein 2 gene, BMP2, lies in this region. We have screened 40 individuals using single stranded conformational polymorphism, SSCP, for structural variation in the BMP2 gene that codes for the mature protein. No consistent changes were observed. Sequence analysis of these individuals suggests a single nucleotide substitution not detected with SSCP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69058-01 PECR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetic Analysis of PP-1 Components in Insulin Resistant Pimas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Prochazka	Senior Staff Fellow	CDNS, NIDDK
Others:	C. Bogardus	Chief	CDNS, NIDDK
	H. Mochizuki	Special Volunteer	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service, University of Dundee, Scotland (P. Cohen)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	0.2	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Insulin resistance is predictive of the development of non-insulin-dependent diabetes mellitus in Pima Indians and this abnormality is associated with decreased rates of glycogen synthesis in skeletal muscle. In vivo-insulin action in Pimas correlates with activities of glycogen synthase (GYS) and of its regulatory enzyme, protein phosphatase type-1 (PP1), and insulin resistant subjects have decreased activities of glycogen-bound PP1, despite an increased mount of immunoreactive PP1 catalytic subunit. Glycogen-associated PP1 activity is determined by an isoform of the catalytic subunit complexed with the glycogen-targeting regulatory (G) subunit, and structural alterations of either component could result in the biochemical abnormalities observed in insulin resistant Pimas. Three genetically distinct PP1 catalytic subunit isoforms are known (PP1 alpha, PP1 beta, and PP1 gamma), and although PP1 beta is believed to be the predominant glycogen-bound form, PP1 alpha, and PP1 gamma may also contribute to the glycogen-associated PP-1 activity. We have determined the exon-intron structure of the genes of PP1 alpha catalytic subunit (PPP1CA; 7 exons), PP1 beta (PPP1CB, 8 exons), PP1 gamma (PPP1CC, 7 exons), and PP1 regulatory G-subunit (PPP1R3, 4 exons) and analyzed PPP1CB first. No sequence alterations were detected by Southern blot analysis or by single-stranded conformational polymorphism (SSCP) of the 8 exons. We have also isolated and analyzed polymorphic CA-repeat markers at PPP1CB, and PPP1CA, and found no evidence for linkage with fasting insulin or insulin action in vivo. We conclude that genetic variations in PPP1CB or PPP1CA are not the cause of PP1 abnormalities or impaired insulin action in Pima Indians. Similar linkage analyses of (CA)-repeat markers at PPP1CC and PPP1R3 are in progress.

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